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CONSUMO DE VACAS LECHERAS EN PASTOREO: COMPORTAMIENTO INGESTIVO Y REGULACIÓN ENDÓCRINO-METABÓLICA EN EL CORTO PLAZO

por

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RESUMEN

El objetivo del presente trabajo fue evaluar en vacas lecheras lactando el efecto de la dieta y de la paridad en el comportamiento ingestivo y en la regulación endócrino-metabólica del consumo de materia seca (CMS) en el primer evento de consumo diario. Se realizaron dos experimentos en bloques completos al azar. En el primer experimento (E1), 14 vacas multíparas de 150 días en leche fueron asignadas aleatoriamente a dos tipos de dieta: dieta totalmente mezclada (DTM) ofrecida *ad libitum* o pastoreo de avena + concentrado (0,9% PV/día; PAS). En el segundo experimento (E2), 9 vacas multíparas (MUL) y 9 vacas primíparas (PRI) de 75 días en leche, pastorearon juntas una pastura de avena y fueron suplementadas individualmente con dieta parcialmente mezclada (DPM; 1,5% del PV). En ambos experimentos, se registró el comportamiento ingestivo y se estimó el CMS del primer evento y el CMS total. Se determinó la concentración de hormonas y metabolitos en sangre, así como la concentración de metabolitos en hígado (solo para E1), en muestras de sangre y biopsias de hígado, respectivamente, colectadas antes y después del primer evento de consumo. Los datos fueron analizados utilizando el procedimiento MIXED de SAS con medidas repetidas en el tiempo (cuando correspondió) y utilizando el procedimiento GLIMMIX para las variables de comportamiento ingestivo. En el E1, el CMS total fue 19,8% mayor (P = 0,025) en las vacas TMR que en las vacas PAS, y se observó una diferencia numérica (19,2%) en el CMS del primer evento a favor de las vacas DTM. La relación insulina:glucagón y la relación ATP:ADP se incrementaron post-consumo (P < 0,05), mientras que la glucosa en plasma disminuyó (P < 0,05) solo en DTM. En el E2, el CMS total y el CMS en el primer evento fueron 17,5% y 25% mayores en MUL que en PRI (P < 0,05). Esto se asoció a una mayor concentración de NEFA pre-pastoreo y mayor concentración de BHB post-pastoreo (P < 0,05) en MUL que en PRI. La oxidación hepática de nutrientes no parecería jugar un rol principal en la regulación del consuno en el corto plazo en pastoreo. Distinta sensibilidad de los tejidos al BHB y/o NEFA podría estar involucrada en la regulación del CMS entre MUL y PRI.

Palabras clave: evento de consumo, paridad, pastura de avena, oxidación hepática

INTAKE OF DAIRY COWS AT GRAZING: INGESTIVE BEHAVIOUR AND SHORT-TERM ENDOCRINE-METABOLIC REGULATION

SUMMARY

The aim of this work was to evaluate in lactating dairy cows the effect of diet and the effect of parity on feeding behaviour and on endocrine-metabolic regulation of DMI at the first daily feeding event. Two experiments were performed in a randomized block design. In the first experiment (E1), 14 multiparous cows of 150 days in milk were randomly assigned to two diet treatments: TMR offered *ad. libitum* or to graze oat pasture + concentrate (0.9% BW/day; PAS). In the second experiment (E2), 9 multiparous (MUL) and 9 primiparous cows (PRI) of 75 days in milk, grazed together an oat pasture and were individually supplemented with partial mixed ratio (PMR; 1.5% of BW). For both experiments, feeding behaviour was recorded and DMI of the first feeding event and total DMI were estimated. Blood hormones and metabolites concentration were determined as well as liver metabolites (only for E1), from blood samples and liver biopsies, respectively, collected immediately before and after first feeding event. Data were analyzed using the MIXED procedure of SAS with repeated measures (when corresponded) and using GLIMMIX procedure for grazing behaviour variables. In E1, total DMI was 19.8% greater ($P = 0.025$) for TMR than PAS cows, and a numerical difference (19.2%) was observed in DMI of the first feeding event in favor of TMR cows. The insulin:glucagon ratio and ATP:ADP ratio increased post-feeding ($P < 0.05$), while plasma glucose decreased ($P < 0.05$), only in TMR. In E2, total DMI and DMI of the first grazing event were 17.5% and 25% greater for MUL than PRI cows ($P < 0.05$). This was related to greater pre-grazing NEFA and greater post-grazing BHB concentration ($P < 0.05$) in MUL than PRI cows. Hepatic oxidation of nutrients would not play a significant role in grazing short-term DMI regulation. Differences between multiparous and primiparous would be explained by different tissue sensitively of BHB.

Keywords: grazing event, parity, oat pasture, hepatic oxidation

1. INTRODUCCIÓN

1.1. PLANTEO DEL PROBLEMA

En sistemas lecheros pastoriles, el CMS es la principal limitante de la producción de leche (Kolver y Muller, 1998; Bargo *et al.*, 2002). Independientemente del sistema de producción y/o condiciones de alimentación, el CMS se compone por la integración de comidas discretas a lo largo del día, denominadas eventos de consumo (Baile y Forbes, 1974; Gibb *et al.*, 1998). En pastoreo se identifican generalmente tres principales eventos de consumo: al amanecer, cercano al mediodía y previo a la puesta del sol (Gregorini *et al.*, 2006; Sheahan *et al.*, 2013). La intensidad de cada evento (duración x tasa de consumo) varía a lo largo del día, así como también el intervalo entre los mismos (Allen, 2000).

La decisión de cuando comenzar o terminar un evento de consumo es afectada por la integración de múltiples señales en los centros de saciedad del cerebro (Allen, 2000). En pastoreo, estas señales son aún más complejas debido a la interacción pastura-animal (Ginane *et al.*, 2015). De esta manera, el comportamiento ingestivo está afectado por señales externas e internas al animal (Gregorini *et al.*, 2006). El manejo del pastoreo, tal como el tiempo de acceso a la pastura (Kennedy *et al.*, 2009), asignación y disponibilidad de forraje (Chilibroste *et al.*, 2012) han sido reportados como las principales determinantes del CMS. Además, características del forraje tales como concentración de fibra detergente neutro (FDN), digestibilidad de la FDN (Oba y Allen, 1999) y el bajo contenido de materia seca (MS) de las pasturas (Bargo *et al.*, 2002), afectan el CMS por efecto de llenado ruminal. Sin embargo, en pasturas de alta calidad estos factores parecerían no ser los principales determinantes del CMS (Chilibroste *et al.*, 1997; Hills *et al.*, 2015). La osmolaridad y pH ruminal (Grovum, 1995), tasa de generación de productos de fermentación o concentración ruminal de N-NH_3 (Chilibroste *et al.*, 1998; Chilibroste, 1999) también afectan el CMS. Otros factores como la concentración de hormonas y metabolitos en sangre en respuesta a la ingestión de alimento (Sheahan *et al.*, 2013), período de ayuno (Patterson *et al.*, 1998), estado fisiológico animal, el cual define la demanda de nutrientes para la producción de leche (Gibb *et al.*, 1999; Allen, 2014) y/o la

sensibilidad de los tejidos a las hormonas (De Koster y Opsomer, 2013), y los ritmos circadianos (Roche *et al.*, 2008) también influyen en el CMS.

Por otra parte, estudios en vacas lecheras, utilizando DTM con alta concentración energética, sustentan que un evento de consumo puede finalizar debido a señales generadas en el hígado por la oxidación de nutrientes. El propionato generado en el rumen durante un evento de consumo, es rápidamente absorbido por la pared ruminal y alcanza el hígado vía vena porta (Allen y Bradford, 2012). Dependiendo del estado fisiológico de la vaca y la demanda de glucosa por la glándula mamaria, el propiónico puede oxidar acetil-CoA, generando ATP. Este incremento en la carga energética del hepatocito, o cambios en la tasa de producción y utilización del ATP afectan el CMS (Allen, 2014). En pastoreo, si bien el patrón de fermentación es distinto a DTM, pueden alcanzarse altos niveles de propionato con pasturas de calidad (Wales *et al.*, 2000). Sin embargo, en estos sistemas, el efecto de la oxidación hepática en la regulación del CMS durante una comida ha sido poco explorado.

Además, el estado interno del animal juega un rol importante en la percepción del recurso alimenticio y el comportamiento ingestivo en pastoreo (Gregorini *et al*., 2009). Sin embargo, el efecto de la paridad en el estado interno y en la regulación del CMS en el corto plazo también ha sido poco explorado.

En este contexto, este trabajo de Maestría permite incrementar el conocimiento sobre la regulación del CMS y el comportamiento ingestivo de vacas lecheras en pastoreo con distinta paridad y tipo de dieta. Además, podría contribuir al desarrollo de estrategias de alimentación que permitan incrementar la eficiencia del sistema optimizando el CMS según la paridad y el estado fisiológico del animal.

1.2. ANTECEDENTES BIBLIOGRÁFICOS

1.2.1. Control temporal del consumo

El CMS diario está determinado por la sumatoria de eventos de consumo, y depende del tamaño de cada comida individual (duración de cada evento y el volumen consumido) y el intervalo de tiempo entre dichos eventos. Múltiples señales son

integradas en los centros de saciedad del cerebro y traducidas en eventos de consumo o no consumo, lo que ha sido reportado por muchos autores como la regulación multifactorial del consumo (Forbes, 1996). Esto incluye señales que incrementan el hambre (orexigénicas) y señales que incrementan la saciedad (anorexigénicas), y su efecto varía desde minutos y horas (reguladores en el corto plazo) hasta semanas y meses (reguladores en el largo plazo) (Allen, 2014). Los mecanismos reguladores en el largo plazo incluyen aquellos que afectan el mantenimiento del peso vivo (por ejemplo, incremento en los niveles de leptina al incrementar las reservas de grasa corporal, lo cual afecta negativamente el consumo), así como las adaptaciones homeoréticas asociadas a la preñez y lactación (ej. cambios en las concentraciones de hormonas y citokininas en sangre durante el ciclo de lactación). Estos cambios en los estados fisiológicos se caracterizan por diferencias en gluconeogénesis en el hígado, movilización y captura de nutrientes (NEFA, aminoácidos, glicerol) por tejidos extrahepáticos, la capacidad secretora del tejido mamario y la secreción y sensibilidad de los tejidos (Allen, 2014). En el corto plazo, el consumo se asocia a la motivación a consumir, la cual depende de cuan hambriento se encuentre el animal y del incentivo a consumir. La primera está asociada a factores nutri-fisiológicos (Gregorini, 2011), es decir, depende de las características de la dieta, que afectan la distensión del aparato digestivo, oxidación de los nutrientes, liberación de péptidos y hormonas y su respuesta varía dependiendo del estado fisiológico del animal (Allen *et al.*, 2009). Mientras que el incentivo a consumir se asocia a ciertos estímulos/incentivos externos (Toates, 2002), relacionados al manejo, al clima, al fotoperíodo, entre otras.

Por lo tanto, el consumo es determinado por la interacción de mecanismos con diversos efectos temporales, donde probablemente la interacción entre el aumento y la disminución de las señales orexigénicas y anorexigénicas esté determinando el inicio o cese de un evento de consumo y el tiempo entre eventos (Allen, 2014).

1.2.2. Regulación en pastoreo

El CMS (g MS/día) de animales en condiciones de pastoreo es generalmente expresado como una función simple producto del tiempo efectivo de pastoreo

(minutos/día), tasa de bocado (bocados/minuto) y masa de bocado individual (g MS/bocado) (Allden y Whittaker, 1970). El tiempo destinado al pastoreo diario es el producto de la duración de los eventos de consumo por el número de eventos que ocurren en el día (Gibb *et al*., 1998). El patrón temporal de pastoreo está modulado por el efecto de los ritmos circadianos (Chilibroste *et al.*, 2015), concentrando la mayor actividad de pastoreo en torno al amanecer y al atardecer, siendo ésta última sesión más larga e intensa (Sheahan *et al.*, 2013). La situación a la cual se enfrenta normalmente la vaca lechera de traslado a la sala de ordeñe y generalmente largo tiempo de espera en la misma, generan períodos de ayuno que modifican el patrón normal de comportamiento, situando los principales eventos de consumo luego de cada ordeñe (Chilibroste *et al*., 2007).

Sin embargo, la función simple definida anteriormente lleva implícita diversos mecanismos complejos de interacción planta-animal que aún no han sido totalmente comprendidos. Dentro de una escala temporal-espacial, de tiempo de acceso y potrero, los vacunos en pastoreo definirán cuando comenzar y cuando finalizar la sesión de pastoreo, con qué frecuencia realizar los eventos de consumo y como se distribuirán a lo largo del día, modificando la dimensión de sus bocados, la tasa de bocado y los tiempos de pastoreo, rumia y descanso, en función de varias señales. A modo de síntesis, las principales señales involucradas en la regulación en el corto plazo se pueden agrupar en las que provienen de la dieta pastoril consumida (asociadas al llenado físico del rumen, digestibilidad de los nutrientes, productos de la fermentación, etc.), las relacionadas al estado fisiológico del animal (asociado a los requerimientos y cambios metabólicos), y a las interacciones generadas entre las distintas señales relacionadas al hambre y saciedad (Decruyenaere *et al.*, 2009; Gregorini *et al.*, 2011; Chilibroste *et al.*, 2015).

1.2.2.1. Características relacionadas a la dieta pastoril

En pastoreo, el llenado físico del rumen ha sido reportado como uno de los principales factores que incide en la regulación del consumo voluntario en el corto plazo (Gregorini *et al.*, 2009). Diversos estudios enfocados en la regulación física del consumo, utilizando diferentes mecanismos de llenado artificial (globos con agua,

globos con aire, pelotas de tenis, fibras artificiales, etc.) han reportado una disminución en el CMS por estimulación de los tensoreceptores ruminales. Gregorini *et al.* (2009), variando el nivel de contenido ruminal de vacas fistuladas previo al pastoreo observaron una tendencia a menor tasa de consumo (g de MS por unidad de tiempo) con el aumento de llenado del rumen. Otros autores reportan una disminución en el consumo a bajos contenidos de MS del forraje (menos de 20%) a pesar de su alta digestibilidad, debido al alto contenido de agua intracelular (Cabrera Estrada *et al.*, 2004). Las dietas altas en FDN de lenta degradación y bajas en proteína requieren mayor tiempo de retención ruminal, afectando también el consumo voluntario (Oba y Allen, 1999). La suplementación lipídica puede reducir la tasa de pasaje del alimento por efectos asociativos en la reducción de la digestibilidad de la FDN (Allen, 2000; Barletta *et al.*, 2016). Por lo tanto, aquellos factores que afecten el nivel de llenado ruminal y la tasa de pasaje pueden modificar el comportamiento en pastoreo, afectando el consumo voluntario (Allen *et al.*, 2005). Por otro lado, otros autores sostienen que en pastoreo de pasturas de alta calidad el efecto de llenado ruminal no sería la principal determinante de regulación del consumo en el corto plazo (Chilibroste *et al.*, 2000).

Productos de la fermentación ruminal (concentración de N-NH_3 y ácidos grasos volátiles (AGV)) también han sido estudiados como señales hipofágicas de regulación del consumo. Distintos trabajos han sido realizados utilizando infusiones de AGV (Forbes, 1996; Forbes 2007; Oba y Allen, 2003a), dietas con distinta degradabilidad ruminal (en confinamiento principalmente) (Bradford y Allen, 2007) e infusiones de amonio (Oba y Allen, 2003b). Los resultados sugieren que a mayor nivel de almidón en la dieta aumenta la proporción de AGV y la relación acético/propiónico disminuye, pudiendo aumentar la hipofagia al igual que las altas tasas de producción de N-NH_3. Chilibroste *et al.* (1998) utilizando vacas fistuladas en pastoreo observó un aumento en la concentración ruminal de AGV a medida que avanza la sesión de pastoreo, y la misma fue menor a mayor ayuno, probablemente por menor masticación del forraje durante la ingesta. El mismo perfil fue observado para la concentración de N-NH_3, aunque el pico se alcanza antes que el pico de AGV

(Chilibroste, 1999). Estos mecanismos de regulación no son independientes, sino que la combinación de factores aumenta el efecto hipofágico (Mbanya *et al.*, 1993), y la magnitud del mismo dependerá del proceso ingestión-digestión (Chilibroste *et al*., 2007).

1.2.2.2. Características relacionadas al estado fisiológico del animal

Si bien las características de la pastura ejercen un efecto importante sobre el comportamiento ingestivo de los animales, éste se ve modificado por cambios en el estado fisiológico de los mismos. Esto puede observarse claramente en los resultados obtenidos por Gibb *et al*. (1999), quienes reportan mayores tasas de consumo (1,4 vs 1,2 kg/MS/h) y mayor tiempo de pastoreo (582 vs 451 min. por día) en vacas lactando que en vacas secas. También se han reportado diferencias en tasa de consumo y tiempo de pastoreo entre paridad. Las vacas primíparas consumen a menor tasa que las vacas multíparas en similar tiempo de consumo (Peyraud *et al*., 1996; Phillips y Rind, 2001).

Los diferentes estados fisiológicos por los cuales transita una vaca lechera en producción (parto, lactancia y sus etapas, gestación, etc.) se caracterizan por diferencias en las concentraciones sanguíneas y sensibilidad de los tejidos a las diferentes hormonas que afectan muchos procesos metabólicos, incluyendo gluconeogénesis y movilización y captura de nutrientes por los tejidos (Allen, 2014). Además, el perfil metabólico de vacas en lactación difiere entre paridad (Santos *et al*., 2001; Meikle *et al*., 2004, Nikkhah, 2012). El periparto es un período de grandes desbalances metabólicos, donde el CMS se restringe al mismo momento que los requerimientos se incrementan. Esto genera una situación de movilización de reservas corporales y de balance energético negativo (BEN) (Meikle *et al.*, 2013), el cual ha sido reportado ser más severo en vacas primíparas que en multíparas bajo condiciones de pastoreo (Meikle *et al.*, 2004), debido en parte a la dificultad de adaptación al mismo, aún en condiciones de alta oferta de forraje (Chilibroste, 2012). En inicio de lactancia se produce un desacople en el eje somatotrófico, producto de la disminución de los receptores de GH en el hígado, lo cual genera una baja concentración de IGF1 circulante y por lo tanto un feed back negativo a la secreción

de GH. En parte, este desacople está explicado por la disminución de la concentración de insulina en sangre (Meikle *et al.*, 2013). Esta situación favorece la captura de glucosa por la glándula mamaria y el catabolismo periférico, generando un gran flujo de NEFA al hígado, para soportar la lactancia en una etapa donde el consumo está restringido (Meikle *et al.*, 2013). Dicha movilización de reservas aumenta a mayor condición corporal (CC) preparto, generando un mayor efecto hipofágico (Remppis *et al.*, 2011; Weber *et al.*, 2013). Esta pérdida de CC es, en general, más abrupta en primíparas que en multíparas, principalmente por el menor consumo en pastoreo, generando un aumento más marcado en la concentración de NEFA en plasma (Meikle *et al.*, 2004). A su vez, al aumentar la concentración de NEFA circulante aumenta su captación por el hígado y por lo tanto su oxidación, produciéndose un incremento en la concentración de acetil-CoA y en la exportación de cetonas (Reynolds *et al.*, 2003). Este aumento en los cuerpos cetónicos podría explicar parte de la reducción en el consumo en lactancia temprana actuando a nivel de los centros regulatorios en el cerebro (Kuhla, 2012), por otro lado, la reducción en la exportación de cuerpos cetónicos, mediante la oxidación de acetil-CoA en el hígado podría también estar afectando dicha regulación.

1.2.2.3. Interacciones entre las distintas señales relacionadas al hambre-saciedad

El inicio o cese de una sesión de consumo está mediada, entre otros, por la distensión ruminal, concentración de metabolitos y señales hormonales emitidas por diferentes órganos periféricos (como por ejemplo hígado, páncreas y tracto gastrointestinal). Los factores descritos anteriormente, relacionados a la dieta y al estado fisiológico, que actúan en la regulación de corto plazo, no tienen efectos independientes, sino que interaccionan entre sí. Por ejemplo, la introducción intraruminal de un globo inflado a un volumen de 10 litros en vacas en inicio de lactancia restringió fuertemente el CMS, sin embargo, en lactancia tardía cuando los requerimientos son menores, el efecto físico de llenado no sería tan determinante en la regulación del CMS (Decruyenaere *et al.*, 2009). Resultados obtenidos en diferentes experimentos también sugieren una interacción entre estado de lactancia y productos de la fermentación. En este sentido, Stocks y Allen (2012) reportaron una reducción en el

CMS y de energía metabolizable (EM) con infusiones de propionato en lactancia temprana en relación a infusiones de acetato. Oba y Allen (2003a), también reportan una reducción en el CMS, tanto en lactancia temprana como en lactancia media a mayor relación propionato/acetato infundida. Sin embargo, el comportamiento ingestivo y la magnitud del efecto, en este trabajo, mostró que las vacas en lactancia temprana redujeron el tamaño de las comidas y no variaron el tiempo entre comidas, mientras que las vacas en lactancia media redujeron tanto la frecuencia entre comidas como el tamaño.

1.2.3. Teoría de la regulación hepática del consumo

Evidencias experimentales, generada en monogástricos principalmente, sustentan que el consumo en el corto plazo es regulado por la oxidación de compuestos en el hígado. En la década del 50´, se postula la teoría glucostática propuesta por Mayer (1953) en la cual el animal, mediante el consumo, intenta mantener un nivel estable de glucosa en sangre regulado por el sistema nervioso central. Más adelante, Russek (1963) observa cambios en el comportamiento en perros asociado a glucoreceptores en el hígado, introduciendo la idea de que el hígado también está involucrado en la regulación del consumo. Posteriormente, Di Bella *et al.* (1981) bajo la hipótesis de que el hígado actuaba termogénicamente, calentando el hígado de ratas artificialmente observaron un aumento en la actividad de masticación y una reducción en el CMS, sugiriendo que termoreceptores en el hígado podrían jugar un rol en la regulación del consumo. Estudios posteriores en el mismo modelo animal demostraron que varios compuestos metabolizados en el hígado deprimían el consumo y que esto se revertía seccionando los nervios que unen el hígado con el cerebro (Langhans *et al.*, 1985; Anil y Forbes, 1988). El hígado está conectado con los centros de saciedad del cerebro vía nervio vago y la tasa de disparo del mismo (determinada por la oxidación de compuestos) se asocia a los eventos de hambre o saciedad (Niijima, 1969; Langhans *et al.*, 1985).

Sin embargo, el efecto de la glucosa en la regulación del consumo no se observó en rumiantes. Trabajos realizados con infusiones ruminales y cerebroventriculares de glucosa en cabras, ovejas y vacas demostraron no tener efecto en la regulación del

consumo voluntario (Seoane *et al.*., 1972; Allen, 2000). Esto se debe a los diferentes compuestos que son oxidados en el hígado de rumiantes y de no rumiantes, ya que la fermentación ruminal altera el tipo y patrón de absorción de los nutrientes. La glucosa que es captada por el hígado de rumiantes es mínima debido a que en el rumen la misma se degrada a distintos AGV. Por otro lado, la hexokinasa, necesaria para la activación de la glucosa en el metabolismo, presenta baja actividad, determinando que el hígado sea un órgano productor de glucosa a partir de precursores como propionato, lactato, amino ácidos y glicerol (Allen, 2014).

Por otro lado, infusiones de propionato en vena mesentérica (Elliot *et al.*, 1985), vena porta (Anil y Forbes, 1980) y en rumen (Oba y Allen, 2003c) provocaron una disminución en el CMS, y el efecto fue revertido seccionando el nervio vago (Anil y Forbes, 1988). La reducción en el CMS fue menor cuando se realizaron infusiones de acetato y butirato (Anil y Forbes, 1980; Elliot *et al.*, 1985; Oba y Allen, 2003c) y en todos los casos se incrementó la hipofagia con el aumento de la dosis infundida (Oba y Allen 2003c; Stocks y Allen, 2012). El efecto hipofágico causado por el propionato puede ser explicado por la rápida absorción y alta actividad de la enzima propionil CoA sintetasa en el hígado de rumiantes. El acetato es metabolizado principalmente en tejidos extrahepáticos, determinando una baja actividad de la acetil-CoA sintetasa en el hígado, mientras que el butirato es metabolizado principalmente en la pared ruminal a beta hidroxibutirato (BHB), lo cual puede explicar el menor efecto hipofágico de estos compuestos (Allen, 2014). El efecto asociado al tipo de infusión (ácidos o sales) fue evaluado por Oba y Allen (2003d), quienes trabajando con infusiones de ácido propiónico y sales de propionato no encontraron un efecto directo del pH ruminal en el comportamiento ingestivo de vacas lecheras.

El propionato es utilizado en el hígado para gluconeogénesis (consumiendo ATP) o es oxidado en el ciclo del ácido tricarboxílico generando ATP (Allen y Bradford, 2012). La concentración de ATP en el hígado se asoció al comportamiento ingestivo (Ji y Friedman, 1999). Rawson y Friedman (1994) capturando fosfato inorgánico mediante la adición de otras moléculas para reducir la disponibilidad interna en el animal, observaron un estímulo en el consumo en ratas, mientras que el consumo se

deprimió al agregar fosfato adicional, sugiriendo que el efecto causado no se debe al fosfato *per se*, sino a la molécula de ATP.

1.2.4. Conocimiento sobre la regulación hepática del consumo en vacas lecheras

Como se mencionó anteriormente el propionato es rápidamente absorbido en el rumen y vía vena porta llega al hígado donde es metabolizado. Trabajos realizados con infusiones intraruminales de propionato sugieren una interacción entre el estado de lactancia y nivel de propionato en la dieta con la reducción en el CMS (Oba y Allen, 2003a). Esto puede deberse a los diferentes destinos metabólicos del propionato según la demanda de glucosa del animal y su estado fisiológico. En lactancia temprana existe una alta demanda de glucosa por la mayor producción de leche y por lo tanto una alta desviación de propionato a gluconeogénseis. La alta concentración de acetil-CoA estimula la síntesis de la enzima piruvato carboxilasa, incrementando la formación de oxalacetato a partir de piruvato (Ballard *et al.*, 1968). Esto indica que la síntesis de oxalacetato a partir de propionato también se vería incrementada por anapleurosis. De este modo, el propionato estimula tanto la gluconeogénesis como el metabolismo oxidativo en el hígado simultáneamente, incrementando los niveles de glucosa, reduciendo el pool de acetil-CoA proveniente de los NEFA y por lo tanto incrementando la carga energética en el hepatocito (por ejemplo: mayor concentración de ATP, mayor ATP/ADP) y reduciendo la exportación de cetonas (Allen *et al.*, 2009; Allen y Bradford, 2012).

Piantoni *et al*. (2015) reportan una mayor concentración hepática de acetil-CoA en lactancia temprana, respecto a lactancia tardía, asociado al estado lipolítico, y la variación en dicha concentración estuvo relacionada negativamente con el CMS, medido en un lapso de 4 h. A su vez, Stocks y Allen (2012) reportan un incremento del efecto hipofágico del propionato a mayor concentración de acetil-CoA en el hígado. Otros trabajos realizados con infusiones de propionato asocian la reducción en el CMS con un incremento en la concentración de glucosa en plasma, una disminución en la concentración de BHB, mientras que en la concentración de insulina y NEFA no se encontraron diferencias significativas (Oba y Allen, 2003e). Esta información indicaría que, en lactancia temprana, a mayor concentración de

acetil-CoA en el hígado, mayor es la desviación de propionato a oxidar el pool de acetil-CoA por anapleurosis, observándose en la reducción de su exportación como centonas. A medida que progresa la lactancia los niveles de insulina y la sensibilidad de los tejidos a la misma se incrementa, así como la demanda de glucosa por parte de la glándula mamaria disminuye. Esta señal de buen estatus energético inhibe la gluconeogénesis, derivando más propionato a la oxidación hepática e incrementando la carga energética en el hígado (Allen y Bradford., 2009).

De acuerdo a estos antecedentes, el consumo de energía en rumiantes será incrementado cuando la energía consumida o glucosa producida por unidad de ATP generado en el hígado por unidad de tiempo se maximice (Allen, 2014). Manipulando el patrón de oxidación de los nutrientes en el hígado puede incrementarse la concentración de glucosa e insulina en plasma, disminuyendo la movilización de grasa y el período de tiempo donde se restringe el consumo por oxidación hepática de los NEFA.

1.3. HIPÓTESIS Y OBJETIVOS DEL TRABAJO

1.3.1. Hipótesis

La oxidación de nutrientes en el hígado y las señales endócrino-metabólicas involucradas en la regulación del consumo en el corto plazo en vacas lecheras alimentadas con DTM, están involucradas en la regulación del consumo en el corto plazo de vacas lecheras en pastoreo (Experimento I). Además, el efecto de las señales endócrino-metabólicas en pastoreo varía según la paridad animal (Experimento II).

1.3.2. Objetivo general

Estudiar la regulación del consumo en el corto plazo de vacas lecheras con énfasis en aspectos metabólicos.

1.3.3. Objetivos específicos

Cuantificar señales de la oxidación hepática de nutrientes, pre- y post- primer evento de consumo diario, y relacionarlas con el comportamiento ingestivo de dicho evento, con señales endócrino-metabólicas y con el consumo total diario en vacas con distinta alimentación (DTM vs dieta base pastura). **Experimento I**

Cuantificar el estado metabólico (glucosa, ácidos grasos no esterificados (NEFA), β-hidroxibutirato (BHB) e insulina) antes y después del primer evento de pastoreo diario de vacas lecheras multíparas y primíparas, y relacionarlo con el comportamiento ingestivo y la ingesta diaria de forraje. **Experimento II**

1.4. ESTRUCTURA DE LA TESIS

La estructura central de la tesis consiste en dos artículos científicos. El primero se titula ***"Short-term feed intake regulation of dairy cows fed TMR or grazing forage oat"*** y constituye el segundo capítulo de esta tesis. Este artículo, enviado y aceptado para su publicación en la revista Animal Production Science, tuvo como objetivo principal cuantificar en vacas en pastoreo y alimentadas con DTM, señales hepáticas y metabólicas que pudieran estar involucradas en la regulación del primer evento de consumo diario. Concluimos que la oxidación hepática de nutrientes parecería regular el CMS en el primer evento de consumo en las vacas alimentadas con DTM, mientras que en vacas pastoreando pasturas templadas estas señales no serían los factores dominantes en la regulación de este evento.

El segundo artículo científico se titula ***"Endocrine-metabolic control of first daily grazing meal of lactating dairy cows"*** y constituye el tercer capítulo de esta tesis. El mismo será enviado a la revista "Livestock Science". El objetivo de este estudio fue estudiar el estado interno del animal como fuente de señales que regulan la ingesta a corto plazo, evaluando el estado metabólico (glucosa, ácidos grasos no esterificados (NEFA), β-hydroxybutyrate (BHB) e insulina) en vacas lecheras multíparas y primíparas, antes y después del primer evento de pastoreo diario, y relacionarlo con el comportamiento ingestivo y la ingesta diaria de forraje. Concluimos que el consumo de forraje en el primer evento de pastoreo está vinculado al estado interno animal y que dicho estado está afectado por la paridad. La concentración sanguínea de NEFA previa al pastoreo y la concentración post-pastoreo de BHB del primer evento de consumo diario, parecerían ser señales involucradas en dicha regulación.
En el cuarto capítulo de esta tesis se presenta una discusión general y conclusiones globales del problema aquí estudiado.

2. SHORT-TERM FEED INTAKE REGULATION OF DAIRY COWS FED TMR OR GRAZING FORAGE OAT

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Running Head: Hepatic feed intake regulation in TMR and grazing

2.1. SUMMARY TEXT FOR THE TABLE OF CONTENTS

In pasture-based dairy systems, dry matter herbage intake is the major limitation of milk production, so is very important to know the signals that regulate herbage intake. An unknown question is the role of liver signals in short-term feed intake regulation, particularly in grazing dairy cows. We have discovered that hepatic oxidation does not have a primary role in the cessation of one of the most important feeding-events at grazing, the first one.

2.2. ABSTRACT

The integration of feeding behaviour with hepatic and endocrine-metabolic signals provides insights for better understanding of short-term intake in dairy pasture-based systems. Therefore, the objective was to quantify hepatic and endocrine-metabolic signals before and after the first daily feeding event relating to feeding behaviour in a TMR vs a grazing pasture-based diet. During 15 days of adaptation and five of measurements, 14 multiparous Holstein cows (DIM = 148±12.7; LW = 535±10.9 kg; BCS = 2.8± 0.08 (1 to 5 scale); milk yield = 28.9±3.32 kg) were assigned to two treatments in a randomized block design: PAS = pasture (herbage allowance = 45 kgDM/cow.day; DM = 21%, NEL = 6.7 MJ/kgDM) + concentrate (0.9% of LW) or TMR (55:45 forage:concentrate ratio, as-dry basis; DM = 40%, NEL = 7.2 MJ/kgDM) ad libitum in a free stall facility. The DM intake of the first feeding event, feeding behaviour and total DM intake and milk production, were measured. Blood and liver samples were taken before and after the first feeding event for hormones and metabolites determination. Comparing TMR vs. PAS cows, total DM and NEL intake, milk production and energy balance were greater ($P < 0.05$), eating and rumination activities were lower (9.2%, $P < 0.01$; 2.4%, $P = 0.06$, respectively) and resting activity was greater (11.6%, $P < 0.01$), while duration and DM intake of the first feeding event did not differ. The insulin:glucagon ratio and liver ATP:ADP ratio increased ($P < 0.05$), and plasma glucose decreased ($P < 0.05$) after the first feeding event only in TMR cows, probably due to greater flux of propionate to the liver. A negative correlation between post-feeding liver ATP:ADP ratio and post-feeding liver acetyl-CoA (r = - 0.82, $P = 0.045$) was also observed only in TMR

cows. It is concluded that hepatic and metabolic signals known to support the hepatic oxidation theory in TMR fed appear not to affect the cessation of the first feeding event in mid-lactation cows grazing a pasture-based diet. Further research is required to relate intake rate, flux of nutrients to liver and its response in hepatic metabolism in grazing dairy cows.

Keywords: Feeding behaviour, Hormones, Dairy pastures

2.3. INTRODUCTION

In pasture-based dairy systems, dry matter (DM) intake is the major limitation of milk production (Kolver and Muller, 1998; Bargo *et al*., 2002). However, independent of the production system and feeding conditions, dairy cows consume individual and discrete meals throughout the day, called also feeding and/or grazing events (Forbes, 1995; Gibb *et al*., 1998). At pasture, three main feeding events are generally observed close to sunrise, in the afternoon and prior to sunset (Gregorini *et al*., 2006; Sheahan *et al*., 2013a). The intensity of each feeding event (intake rate and length of the feeding event) can change throughout the day as well as the interval between them, affecting total DM intake and therefore, milk production (Allen, 2000).

The decision of when to start or finish a feeding event is affected by the integration of internal and external signals of the animal in the brain feeding centers (Allen, 2000; Gregorini *et al*., 2006). In pastures, this process is even more complex, and is affected by grazing management. Access time to pasture (Kennedy *et al*., 2009), herbage allowance and herbage mass (Chilibroste *et al*., 2012), has been reported as the main factors affecting feed intake. Moreover, herbage characteristics, such as neutral detergent fiber (NDF) concentration and its digestibility (Oba and Allen, 1999) and DM content of pasture (Bargo *et al*., 2002), have been reported to affect feed intake due to effects on rumen fill. However, in highly digestible pastures these factors are not be the main determinant of feed intake (Chilibroste *et al*., 1997; Hills *et al*., 2015). Osmolarity and pH (Faverdin, 1999), rate of generation of fermentation products or N-NH3 in the rumen (Chilibroste *et al*., 1998; Chilibroste, 1999) play roles in regulation of feed intake. In addition, feed intake is also regulated by changes in circulating concentrations of hormones and metabolites in response to the ingestion and digestion of feed (Sheahan *et al*., 2013a), fasting period (Patterson *et al*., 1998; Chilibroste *et al*., 2007) or the physiological state of the animal. These factors define the demand of nutrients for milk production (Gibb *et al*., 1999; Allen, 2014) and the sensitivity of tissues to hormones. Moreover, according to the optimal theory of grazing, cows optimize their input and output, by fulfilling their energy and

nutrient requirements at the lowest cost in energy and time spent grazing (Phillips, 2002).

On the other hand, researches on the use of total mixed ration (TMR) diets with high proportion of fermentable starch show that a feeding event could be concluded by signals generated in the liver by oxidation of fuels (Allen *et al*., 2009, Allen, 2014). The production of ATP within meals is affected by the rate of production of anapleurotic fuels (as propionate), and contents of acetyl CoA and reducing equivalents in the liver, and it is highly variable by physiological state and glucose demand of the cow and by the diet (Allen and Piantoni 2013). The metabolic signals from fuel oxidation likely predominate during the transition period (Allen and Piantoni, 2013). However, when glucose demands are increased (peak of lactation), propionate is mainly used for gluconeogenesis, reducing the possibility for propionate oxidation within meals, and extending the length and size of meals. And, in the contrary, when glucose demand is decreased (e.g., past peak of lactation, mid to late lactation), propionate could be oxidized within or during actual meals, resulting in signals of satiety (Oba and Allen, 2003). The higher levels of non-fiber carbohydrates in a TMR diet in comparison to a grass pasture (Waghorn, 2002) and so the rapidly increase of propionate in the rumen might cause a change in the energy status of the liver when a meal is finalized. However, although may vary between sward type, age of regrowth and between and within days (Chilibrsote *et al*., 1998), dairy cows grazing on high digestible grass pastures, can reach high propionate concentration and/or acetate:propionate ratio of 2.6-2.8 in the rumen (Chilibroste *et al*., 1998, Stakelum and Dillon, 2003, Ribeiro Filho *et al*., 2012. According to our knowledge, we have not found studies that have focused on the role of liver signals that may contribute to understand the regulation of short-term feed intake in grazing dairy cows during mid-lactation. We hypothesized that energy status of the liver, determined by the rate of production and utilization of ATP, is associated with short-term feeding behaviour and to the endocrine-metabolic status of dairy cows at grazing. Therefore, the aim of this study was to quantify hepatic oxidation signals before and after the first daily feeding event, and relate them to feeding behaviour

during the event, to blood metabolites and hormones before and after the first daily feeding event and to daily total DM intake in cows fed TMR and at grazing temperate grass pasture.

2.4. MATERIALS AND METHODS

The experiment was conducted at the Experimental Research Station "Dr. M.A. Cassinoni" (EEMAC) of the Facultad de Agronomía (Paysandú, Uruguay, 32º22'52" S, 58º03'10.25" W) during August 2016, at the end of the winter season. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (UdelaR, Montevideo, Uruguay), application number 021130-002616-14.

2.4.1. Experimental design, animals, and treatments

Fourteen Holstein cows were used in a 20-day-trial, 15-day for adaptation to experimental management and diets followed by five days for experimental determinations. Twelve cows were in their second lactation and two in their third lactation. At the start of the experiment, their average (± SD) days in milk (DIM) was 148 ± 12.7, live weight (LW) was 535 ± 10.9 kg, body condition score (BCS, 1-5 scale, 1 = thin and 5 = fat, Edmonson *et al*., 1989) was 2.8 ± 0.08 and milk yield was 28.9 ± 3.3 kg. Cows were selected from an autumn calving herd, blocked by DIM, LW, BCS, number of lactations and previous milk yield and randomly assigned to two treatments: 1) TMR (non-fresh pasture, control) and 2) pasture + concentrate (PAS).

2.4.2. Management and feeding

Cows were milked twice daily, at 4:00 and 15:00 h. After morning milking, they were kept in a pen with access to water until 8:00 h. Access time to treatment was from 8:00 to 14:30 and from 16:00 to 3:30 h. Cows in PAS were fed concentrate (0.9% of LW, Table 1), which was offered in two equal meals at each milking in the milking parlor, and were allowed to graze an oat pasture in one group (*Avena byzantina*). Herbage allowance was 45 kg of DM/cow.day (Table 1). Pasture was offered in daily strips marked off by an electric fence. Fresh daily strips were

accessed in the morning and none were re-grazed during the experiment. The forage availability was estimated to determine herbage allowance, using the technique described by Haydock and Shaw (1975), cutting grass at ground level in a 30 x 30 cm frame, with electric garden hand shears (combined shears GSL35 Black&Decker) and using a rising plate meter (Mattiauda *et al.*, 2013). Oat pasture was sown in March 2016 with 120 kg/ha of seed and fertilized with 46 kg of N in April and June. Cows in the TMR treatment were individually housed in a free stall facility (wood-frame barn) and fed *ad libitum* TMR (Table 1) distributed once daily in the morning.

Table 1. Ingredients and nutrient composition (% as-dry basis) and estimated net energy for lactation (NE_L, MJ/kg DM) of experimental diets

Treatments: TMR, total mixed ration; PAS, pasture plus concentrate at 0.9% of LW/day. DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; NE_L, net energy for lactation estimated according to NRC 2001

	Treatments		
	TMR	PAS	
Item		Concentrate	Pasture
Ingredients (%)			
Corn silage	42.6		
Lucerne hay	8.5		
Sorghum grain	14.8		
Corn grain	5.8	32.0	
Barley grain	8.8	31.0	
Soybean meal	11.6	32.0	
Sunflower grain	6.4		
Insalmix premium	1.2		
Salt (NaCl)	0.3	1.0	
Compound salt		4.0	
Analyzed composition (%)			
DM	40.0	86.9	21.1
OM	93.3	91.2	89.8
Starch[A]	29.8	40.1	
NDF	40.2	30.0	46.9
ADF	20.8	10.2	28.2
CP	14.4	16.5	14.8
EE	5.2	3.0	3.5
NE_L (MJ/kg DM)	7.2	7.4	6.7

[A]Starch: estimated by Dairy One Interactive Feed Composition Libraries

2.4.3. Measurements and sample analyses

2.4.3.1. Milk production, live weight and body condition score

During the five days of measurements, individual milk yields (kg) were recorded at each milking (Waikato MKV® Milk Meter, Hamilton, NZ). Milk fat, protein, and lactose concentrations were determined from one successive AM and PM milk sample taken on the third day (MilkoScan® Foss FT2, Hillerød, Denmark). Fat corrected milk yield (4%, FCM) was calculated using the equation of Tyrrell and Reid (1965).

2.4.3.2. Animal behaviour

Through the first three consecutive days of the measurement period, animals eating, ruminating or resting (not eating or ruminating) were recorded. Cows were observed every 5 min during diurnal access time to treatment. Time spent per activity (min) was calculated assuming that the activity recorded was maintained during the 5 min until the next observation. Length of the first daily feeding event and length of the first daily non-eating event were calculated when the activity was maintained for at least two consecutive recordings.

2.4.3.3. Total DM and energy intake estimation

The total DM intake was estimated during the experimental period using Cr_2O_3 as an external indigestible faecal marker to estimate faecal production (Peyraud, 1998) together with acid insoluble ashes (AIA) in faeces and diet (Sales and Janssens, 2003) as an internal marker. All cows were dosed twice daily, after each milking, for 12 consecutive days (seven days for adaptation and regulation of marker excretion flow and five days for faecal collection) with a bolus of paper containing 7.5 g of Cr_2O_3 (60% purity). After dosing, animals were observed to ensure that there was no regurgitation. Faecal grab samples were collected from the rectum of each cow twice daily after milking. Sward grazing horizon was representatively sampled using electric garden hand shears before first feeding event, from day 7 to 11 during the intake measurement period. Concentrate and TMR samples were collected from each

feeder immediately after feeding during the intake measurement period as well as feed refused. All samples were frozen at -20 □C until dried at 60 □C for 48 h and composited. The DM concentration was determined by drying at 105 □C for 24 h. The ash was determined by combustion in a muffle furnace at 300 □C for 5 h. The organic matter (OM) was determined by mass difference among DM and ash. The total nitrogen was assayed using the Kjeldahl method (Method 984.13; AOAC 2000) and expressed as crude protein (CP, nitrogen x 6.25). The ether extract (EE) was determined using Soxhlet extraction (Method 920.39; AOAC 2000); samples were packed in cartridges of filter paper and the extraction lasted 16 h using petroleum ether. The content of AIA was determined according to Tejada de Hernandez (1983) and NDF and acid detergent fiber (ADF) as described by Van Soest *et al.* (1991), except that the samples were weighted into filter bags and treated with neutral detergent solution that included heat-stable amylase in ANKOM equipment (ANKOM Technology, Macedon NY, USA), and expressed as ash-free residues. Starch concentration was estimated by Dairy One Interactive Feed Composition Libraries. The Cr_2O_3 of faecal samples was determined as described by (Parker *et al.*, 1989).

The total DM intake of TMR treatment, pasture DM intake of PAS treatment and total DM intake of PAS treatment were calculated as:

Total DM intake of TMR treatment (kgDM/cow.day) = $(F * [AIA_F]) / [AIA_{TMR}]$

Pasture DM intake (kgDM/cow.day) = $\{(F * [AIA_F]) – (C * [AIA_C])\} / [AIA_{PASTURE}]$

Total DM intake of PAS treatment (kgDM/cow.day) = C intake + Pasture DM intake

where F = faecal production (kg DM); C = concentrate (kg DM); $[AIA_F]$, $[AIA_{TMR}]$, $[AIA_C]$ and $[AIA_{PASTURE}]$ = concentration of insoluble ashes (%) in faeces, TMR, concentrate, and pasture, respectively.

Net energy requirements for maintenance and lactation (NE***L***, MJ) calculations were based on NRC (2001). The NE***L*** concentration of TMR, pasture and concentrate

were estimated to calculate total NE***L*** intake. Daily energy balance was expressed as an amount of NE***L*** requirements satisfied by NE***L*** intake.

2.4.3.4. First feeding event intake and intake rate

First feeding event intake (kgDM) and intake rate (gDM/min) were estimated at the first daily feeding event (after morning milking) during two consecutive days. For PAS, DM intake was calculated by weighing each cow pre- and post- voluntary cessation of a grazing bout (post-feeding) and corrected for one hour of insensible weight loss, according to the procedure described by Penning and Hooper (1985). Before grazing, animals were fitted with faecal and urine collecting bags to avoid excreta losses. Cows were weighed using a precision balance (accurate to 50 g) in a location protected from wind, 100 m away from the paddock. Three measurements per second were recorded using a complement of Windows (Hyper Terminal private edition v.7.06 electronic Download) until a measurement was repeated at least 10 consecutive times. The LW was calculated as the mode (the most frequent value) of the recorded data. Animals were familiarized with harnesses, faecal and urine collecting bags and the presence of observers during the adaptation period. The intake rate was calculated by dividing DM intake by feeding event duration (min). The DM of pasture consumed was estimated from hand clipping samples of each cow (Jones and Moseley, 1993). For TMR cows, DM intake was measured by the difference between feed offered and feed remaining, and intake rate was calculated as described before. The DM of feed consumed was estimated from representative samples of each feeder taken before DM intake estimation. To estimate NE_L intake at the first feeding event, NE_L of TMR and hand clipped samples were estimated as described for total intake. Besides, first feeding event DM intake / total DM intake ratio (%) and first feeding event NE_L intake / total NE_L intake ratio (%) were calculated.

2.4.3.5. Blood and liver sampling and analyses

Pre-feeding blood and liver sampling of the first daily feeding event were taken the day before the measurement period, and post-feeding samples were taken at the precise moment when each cow had voluntarily finished its first feeding event on the

last day of measurements in order not to interfere with normal behaviour. Blood samples were collected by venipuncture of coccygeal vein into two vacuum tubes, one for serum (BD Vacutainer® REF 367820), and the other for plasma containing sodium fluoride and potassium oxalate (BD Vacutainer® REF 367922) as a glycolytic inhibitor. Both tubes were centrifuged (2000 x g for 15 min at 4 □C) within 2 h after collection and plasma and serum were stored at −20 □C until analyzed. After blood collection, liver biopsies (500 mg) were obtained using a 14-gauge biopsy needle (TruCore®-II Biopsy Instrument; The Hague, The Netherlands) as described by Carriquiry *et al.* (2009). Liver samples were immediately frozen in liquid nitrogen and stored at −80 □C until analyses.

Each metabolite and hormone was determined in a single assay. Plasma glucose was determined bet alorimetric assays on Vitalab Selectra II autoanalyzer (Vital Scientific, Dieren, The Netherlands) using a commercial kit (BioSystems S.A. Costa Brava, 30. 08030 Barcelona, Spain). Serum non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations were analyzed by spectrophotometry using commercial kits: HR Series NEFA-HR (2), Wako and BHB A25-Biosystem Cod: 12525, respectively. Intra-assay CV for all determinations was less or equal to 10%. Serum concentrations of insulin were measured using an immunoradiometric assay (IRMA) with a commercial kit (DIA source INS-IRMA Kit) previously used in cattle (Astessiano *et al.*, 2015). The assay detection limit was 1.518 μIU/mL, and intra-assay CV for control 1 (23.4 μIU/mL) and 2 (76.3 μIU/mL) were 6.2 and 2.2%, respectively. Serum glucagon was measured using a RIA kit (#GL-32K, Millipore, USA) specific for glucagon in serum or plasma in most mammals (Sheahan, 2014). Intra-assay CV for control 1 (62 pg/mL) and control 2 (109 pg/mL) were 9.7 and 9.1%, respectively.

In the liver samples, ATP, ADP and acetyl-CoA concentrations were determined using commercial kits from Abcam (ATP assay kit, Colorimetric/Fluorometric, ab83355; ADP assay kit, Colorimetric/Fluorometric, ab83359 and PicoProbe Acetyl CoA Assay Kit, ab87546, respectively) according to the manufacturer's instructions. The ATP and ADP liver tissue homogenates absorbance were colorimetric measured

using a Thermo Scientific™ Multiskan GO and the fluorescence signal of liver acetyl-CoA tissue homogenate was measured using a Thermo Scientific™ Varioskan Flash™. The relative concentrations were normalized with the fresh tissue mass. In the case of ATP and acetyl-CoA measurements, homogenates were deproteinized using perchloric acid 2-4 N and neutralized with potassium chloride 2 M.

2.4.4. Statistical analyses

All statistical analyses were performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), except daily feeding behaviour, which was done using the GLIMMIX procedure assuming a binomial distribution and estimating the probability of occurrence of the different activities. Residuals were tested for normality distribution using the Shapiro-Wilk test (PROC UNIVARIATE statement).

Total DM intake, components of energy balance, daily milk and milk constituents yield, milk composition, first feeding event behaviour variables were analyzed by the mean of each individual variable using ANOVA. The model included treatment and block as fixed and random effect, respectively.

Model: $Yij = \mu + Ti + Bj + Eij$

where μ = mean; Ti = treatment (i = 1 to 2); Bj = block (j = 1 to 7); and Eij = residual error term.

Blood hormone and blood and liver metabolite concentrations were analyzed with repeated measurements using the following model:

$$Yijl = \mu + Ti + Bj + Eij + Hl + (T*H)il + dijl$$

Where μ = mean; Ti = treatment (i = 1 to 2); Bj = block (j = 1 to 7); Eij = residual error term; Hl = moment of sampling (pre- and post-feeding); (TxH)il = treatment x moment of sampling; and dijl = residual error of the repeated measure.

The model included treatment and moment of sampling as fixed effects, and block as random effect using the first order autoregressive (AR1) as covariance structure. To

improve the accuracy of the models, LW and BCS were tested as covariates specific to the variables being analyzed. Tukey–Kramer tests were conducted to analyze differences between groups ($\alpha = 0.05$). For each treatment, the relationship between first feeding event behaviour variables and pre - and post-feeding metabolic variables were analyzed by Pearson correlations using PROC CORR (SAS Inst. Inc.). For all results, means were considered to differ when $P \leq 0.05$, and trends were identified when $0.05 < P \leq 0.10$. Data are presented as least square means ± pooled standard errors.

2.5. RESULTS

2.5.1. Total dry matter intake, energy balance, milk yield and milk composition

Data of total DM intake, energy balance and milk yield and composition for TMR and PAS treatments are shown in Table 2. Cows fed TMR consumed more ($P < 0.05$) DM and more NE_L than PAS cows (19.8% and 25.9%, DM and NE_L, respectively) with lower ($P < 0.001$) NE_L maintenance requirements. Milk yield was also greater for TMR than PAS cows ($P < 0.001$) with greater lactose content ($P = 0.014$) and without differences in fat and protein content. These differences were also observed in NE_L output for lactation, which was greater ($P = 0.001$) in TMR than PAS cows. The NE_L balance was positive in both treatments and 17% greater ($P = 0.05$) in TMR than PAS cows.

2.5.2. Daily feeding behaviour and first feeding event behaviour

Results describing daily feeding and first feeding event behaviour are shown in Table 2. The probability of finding cows eating during the observation time was greater ($P < 0.001$) for PAS than TMR cows, equivalent to 52 min. The number of feeding events did not differ between treatments, determining greater ($P = 0.002$) feeding event duration for PAS than TMR cows. In contrast, the probability of finding cows resting was lower ($P < 0.001$) for PAS treatment, in spite of spending 30 min more ($P < 0.001$) time in the first non-feeding event than TMR cows. This non-feeding event was positively correlated ($r = 0.98$, $P < 0.01$) to the duration of the first feeding event only in PAS cows. In addition, the probability of rumination tended to be greater ($P = 0.059$) for PAS than TMR cows.

Table 2. Effect of diet on total dry matter and energy intake, feeding behaviour and first feeding event behaviour

Values for treatments are least square means (n=14). s.e.m., standard error of means. Treatments: TMR, total mixed ration; PAS, pasture plus concentrate at 0.9% of LW/day. DM, dry matter. NE_L, net energy for lactation estimated according to NRC 2001; NE_L balance: amount of NE_L requirements satisfied by NE_L intake

	Treatments			*P*-value
	TMR	PAS	s.e.m	treatment
Total feed intake (kg DM)	23.0	19.2	1.16	0.025
Pasture intake (kg DM)	-	14.1	-	-
Estimated NE_L balance				
Intake (MJ/day)	166.9	132.6	8.53	0.012
Maintenance (MJ/day)	39.7	42.3	0.75	<0.001
Lactation (MJ/day)	99.6	85.8	2.34	0.001
Balance	27.0	3.2	7.59	0.030
Yield (kg/day)				
Milk	31.2	26.6	0.88	0.001
Fat corrected milk (4%)	31.4	27.5	0.83	0.006
Fat	1.3	1.1	0.04	0.045
Protein	1.1	0.9	0.04	0.005
Lactose	1.6	1.3	0.04	<0.001
Total milk solids	3.9	3.3	0.08	0.001
Milk composition (%)				
Fat	4.1	4.2	0.16	0.443
Protein	3.4	3.5	0.07	0.607
Lactose	5.0	4.8	0.07	0.014
Feeding behaviour[A]				
Number of feeding events	5.6	5.8	0.31	0.444
Feeding event duration (min/event)	40.5	48.7	2.53	0.002
First non-feeding event duration (min)	54.6	84.8	13.16	<0.001
Eating time (min)	229	281	13.00	<0.001
Rumination time (min)	156	169	9.00	0.059
Resting time (min)	179	114	10.20	<0.001
First feeding event behaviour				
Intake (kg DM)	4.0	3.3	0.40	0.233
NE_L intake (MJ)	29.6	22.5	2.51	0.074
Feeding event duration (min)	74.2	84.1	7.75	0.137
Intake rate (g DM/min)	54.9	40.2	0.18	0.004
First feeding event intake/total intake (%)	18.1	17.9	2.21	0.940
First feeding event NE_L intake/total NE_L intake (%)	20.2	22.7	1.60	0.174

[A]Daily feeding behaviour was recorded during 9.4 hours on day-light hours.

When the first feeding event behaviour was considered, NE_L intake tended (P = 0.074) to be greater for TMR than PAS cows. Although neither DM intake nor feeding event duration differed between treatments, the increase in DM intake (+ 0.64 kg) and decrease in feeding event duration (-9.9 min) resulted in a greater (P = 0.004) intake rate of TMR compared to PAS cows. Furthermore, the DM intake tended to be positively correlated to intake rate (r = 0.77, P = 0.071) in PAS cows, while in TMR it was correlated to the duration of the event (r = 0.95, P < 0.01).

2.5.3. Pre- and post-feeding blood and hepatic metabolic variables

No differences between treatments were found in pre- and post-feeding NEFA concentrations. However, as was expected, the concentration of NEFA decreased (P < 0.01) in both treatments after feeding (Figure 1 A). The pre- and post-feeding BHB concentrations were greater (P < 0.05) for TMR than PAS cows and increased (P < 0.05) significantly with feed intake in both treatments (Figure 1 B). Glucose did not differ between treatments in the pre- and post-feeding concentrations and decreased (P < 0.001) with feed intake only in TMR (Figure 1 C). Insulin concentration did not differ neither between treatments nor between moment of sampling (Figure 1 D) while glucagon concentration decreased at the post-feeding sampling only in TMR cows (P < 0.01) thus generating differences (P = 0.048) between treatments post-feeding (Figure 1 E). The insulin:glucagon ratio differed (P = 0.022) between treatments only post-feeding, explained by an increase (P = 0.005) of this ratio in TMR cows after feeding (Figure 1 F).

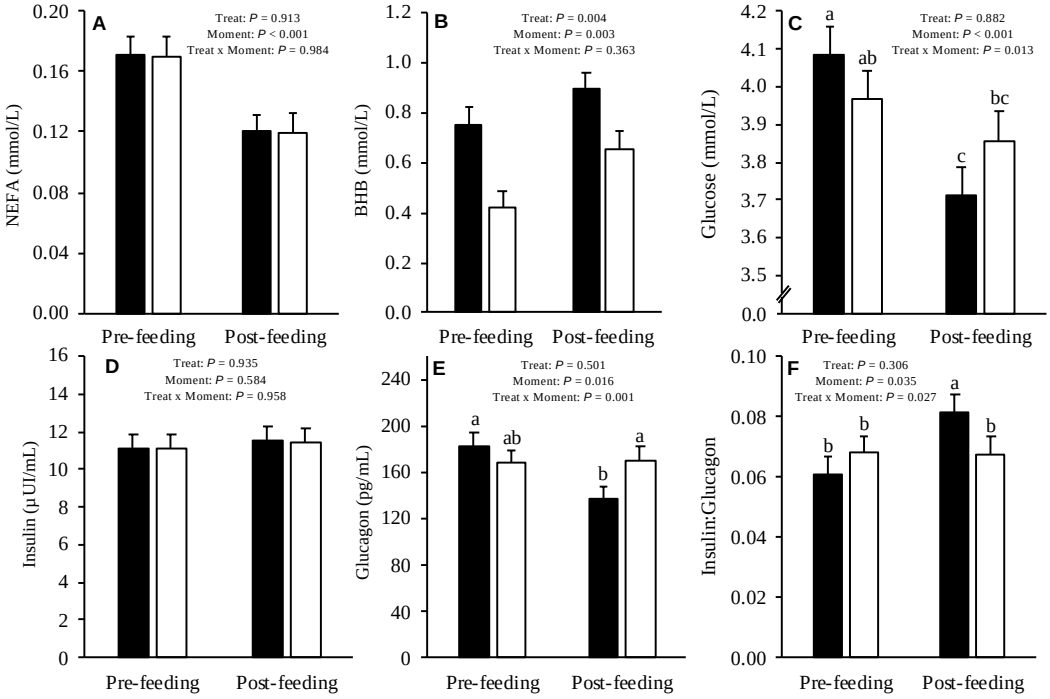

Figure 1. Pre- and post-feeding blood concentration (+ s.e.m) of NEFA (A, mmol/L), BHB (B, mmol/L), Glucose (C, mmol/L), Insulin (D, µUI/mL), Glucagon (E, pg/mL) and Insulin:Glucagon ratio (F) of the first daily feeding event for total mixed ration (TMR, black bar) and pasture (PAS, white bar) treatments. Treat: treatments (TMR and PAS), Moment: Moment of sampling (pre-feeding and post-feeding). Different letters (a,b,c) indicate significant differences (P<0.05) between treatments and moment of sampling.

Hepatic ATP concentration did not differ between treatments or moment of sampling (Figure 2 A). Pre-feeding ADP concentration did not differ between treatments and tended (P = 0.070) to decrease with feed intake only in TMR cows, leading to differences (P = 0.008) between treatments in the post-feeding concentration (Figure 2 B). The inverse response was observed in ATP/ADP ratio, increasing significantly (P = 0.005) after feeding in TMR cows and generating differences (P = 0.001) between treatments in the post-feeding ratio (Figure 2 C). Liver acetyl-CoA concentration was not affected by the first daily feeding event in either treatments, however, the post-feeding concentration of this metabolite in TMR cows was greater (P = 0.007) than PAS cows (Figure 2 D).

For TMR cows, the post-feeding concentration of serum BHB and liver ATP were positively correlated to the first non-feeding event duration (r = 0.78 *P* = 0.040; r = 0.82, *P* = 0.023; respectively).

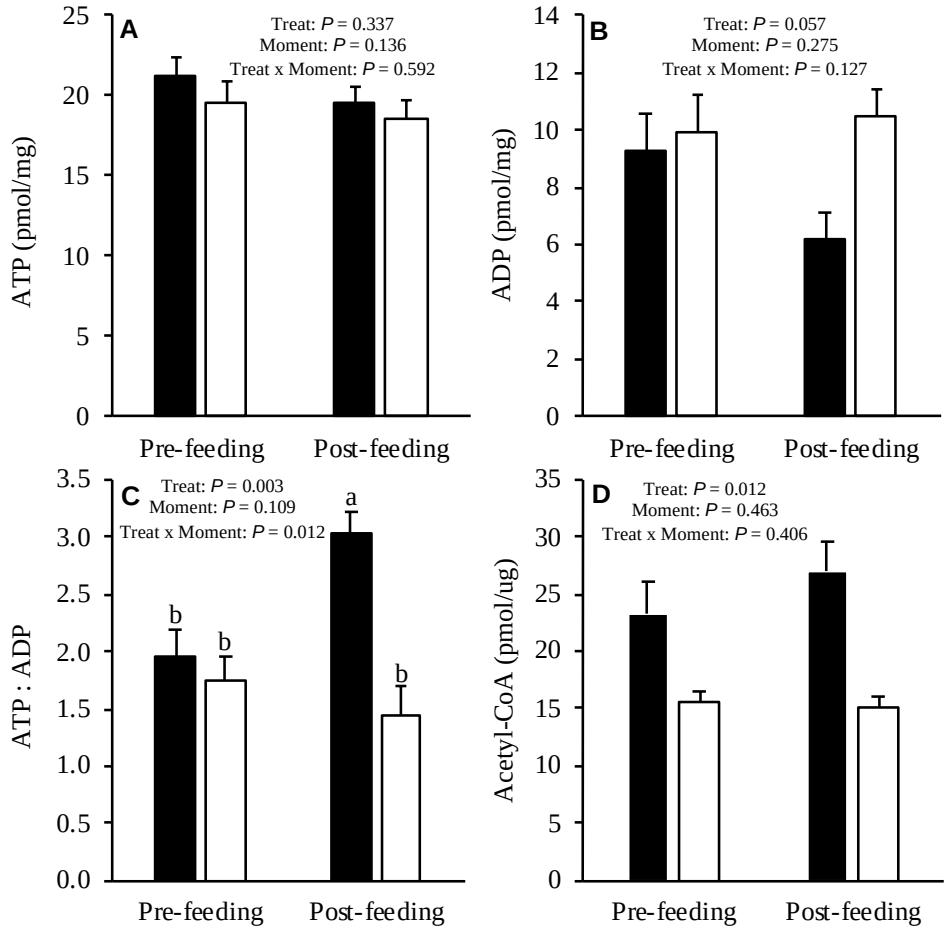

Figure 2. Pre- and post-feeding hepatic concentration (+s.e.m) of ATP (A), ADP (B), ATP:ADP ratio (C, pmol/mg) and Acetyl-CoA (D, pmol/µg) of the first daily feeding event for total mixed ration (TMR, black bar) and pasture (PAS, white bar) treatments. Treat: treatments (TMR and PAS), Moment: moment of sampling (pre-feeding and post-feeding). Different letters (a,b) indicate significant differences ($p<0.05$) between treatments and moment of sampling.

2.5.4. Relationship between behaviour and pre- and post-feeding metabolic variables of the first feeding event

In TMR cows, the first feeding event DM intake was negatively correlated with the decrease in plasma glucose concentration (r = -0.84, *P* = 0.017) and tended to be negatively correlated to pre-feeding liver ATP:ADP ratio (r = -0.74, *P* = 0.059). In addition, post-feeding liver ATP:ADP ratio was positively correlated with the decrease in plasma NEFA concentration (r = 0.76, *P* = 0.045), and was negatively correlated to post-feeding serum insulin:glucagon ratio (r = -0.96, *P* < 0.001), serum BHB (r = -0.75, *P* = 0.052) and liver acetyl-CoA concentration (r = -0.82, *P* = 0.045).

In PAS cows, the first feeding event DM intake was negatively correlated to pre-feeding serum insulin:glucagon ratio (r = -0.87, *P* = 0.024) and tended to be positively correlated to pre-feeding liver ATP:ADP ratio (r = 0.76, *P* = 0.078). The post-feeding liver ATP:ADP ratio was positively correlated with the decrease in serum NEFA (r = 0.96, *P* = 0.008) and the increase in serum BHB (r = 0.96, *P* = 0.009), and tended to be negatively correlated with intake rate (r = -0.83, *P* = 0.078). Additionally, the increase in serum BHB during the feeding event was negatively correlated to intake rate (r = -0.83, *P* = 0.034).

2.6. DISCUSSION

Our hypothesis that energy status of the liver, determined by the rate of production and utilization of ATP, was associated with short-term feeding behaviour and to the endocrine-metabolic status of mid-lactation dairy cows grazing a pasture-based diet, could not be confirmed. Although the type of diet modified feeding behaviour, endocrine and hepatic factors, and total DM intake, the energy status of the liver does not seem to be the main factor affecting short-term feeding behaviour in PAS treatment.

Improvements in DM intake are usually associated with increased milk yield due to an increase in energy intake (Kolver and Muller, 1998; Moallem *et al*., 2000; Bargo *et al*., 2002). The greater total DM intake and milk production observed in TMR cows of the current experiment was consistent with Bargo *et al*. (2002) who compared dairy cows fed TMR and cows at grazing with supplementation. Grazing

as such produces an increase in energy expenditure for more intensive walking required (Bargo *et al*., 2002) compared to animals with feed easily available as a TMR diet (Roca-Fernández *et al*., 2013). So, as expected, the potential increase in maintenance energy of PAS cows and lower NE_L intake compared to TMR, explain a large part of the differences in milk and solids production between dietary treatments.

2.6.1. Daily feeding behaviour and first feeding event behaviour

The pattern of feeding activities observed in the current study was in line with data from other researchers who studied mid lactation cows fed TMR (Mendoza *et al*., 2017) and grazing with supplementation (Kennedy *et al*., 2009). The longest time spent eating by PAS cows was related to longer feeding event duration, in spite of the fact that there were no differences between treatments in the length of the first feeding event. This is also consistent with the lower intake rate observed in the PAS treatment, which supports the longer time needed by grazing dairy cows to meet their energy requirements throughout the day. A possible explanation of this could be the lower DM content of pasture, which takes longer to harvest per unit of DM consumed (Cabrera Estrada *et al*., 2004), longer time for bite manipulation and chewing during ingestion (Thorne *et al*., 2003) and greater searching time (Chilibroste *et al*., 1997; Gregorini *et al*., 2007). The longer duration of the first feeding event compared to the average feeding event in both treatments could have been related to the stimulus of fresh feed delivery for TMR and new pasture strip for PAS cows, as well as to the fasting time during the early morning (Patterson *et al*., 1998; Chilibroste *et al*., 2007; King *et al*., 2016; Miller-Cushon and DeVries, 2017). Interestingly, the DM and NE_L intake in the first feeding event of both treatments, was nearly 20% of the total DM and NE_L intake, emphasizing the importance of the factors regulating the first feeding event intake in the control of total intake (Gill and Rommey, 1994). Additionally, the positive correlations observed between DM intake in the first feeding event with feeding event duration in TMR, and DM intake in the first feeding event with intake rate in PAS show the different ways to optimize feed intake in response to diet and suggest that different signals could be involved in its

regulation (Gill and Rommey,1994).

2.6.2. Control of the first feeding event intake

Previous to the first feeding event, the lack of differences between treatments in most metabolites and hormones is consistent with previous data reported for grazing (Sheahan *et al*., 2013a) and TMR fed dairy cows (Sutton *et al*., 1986; Wylie *et al*., 2008; Nikkhah, 2014). It can probably be associated with the natural fasting during the night and increased rumination activity (Forbes, 1995; Taweel *et al*., 2004) which reduce rumen pool size, increase passage rate and nutrient absorption (Tóthi *et al*., 2003). However, the greater serum concentration of BHB in TMR cows, was not expected and could be associated with greater ruminal pool size, by the greater total DM intake during the day compared to PAS cows, and therefore greater ketone bodies synthesis in the ruminal epithelium (Sheahan *et al*., 2013a; Nikkhah, 2014; Piantoni *et al*., 2015).

Post-feeding, the reduction in serum NEFA concentration in both treatments, showed a change in the energy status from a tissue state of catabolism to anabolism (Lafontan *et al*., 2009). This is also supported by the increase in BHB, which reflects the uptake of ruminal volatile fatty acids during a feeding event (Chilibroste *et al*., 1998). The highest post-feeding BHB concentration reached by TMR cows and the lack of differences in the first feeding event duration and DM intake between treatments, suggest that serum BHB at this concentration would not be a main signal of feeding event cessation. This is consistent with Zarrin *et al*. (2013) who found no effect on feed intake in dairy cows increasing plasma BHB concentration from 0.59 to 1.74 mmol/L by intravenous infusion. However, the higher post-feeding BHB concentration in TMR cows would probably keep the sensation of satiety for longer (Rossi *et al*., 2000) due to the positive correlation observed with the first non-feeding event.

For TMR treatment, the post-feeding decrease in plasma glucose was consistent with other studies (Ametaj, 2009; Iqbal *et al.*, 2012). This variation in glucose concentration could be related to an increase in the insulin:glucagon ratio, which

increases glucose uptake by peripheral tissues (Derno *et al.*, 2013). It is likely that the observed increase in the insulin:glucagon ratio post-feeding may have increased the oxidation rate of propionate in the liver (Derno *et al.*, 2013), which is also supported by the positive correlation between ATP:ADP ratio and insulin:glucagon ratio in this treatment. The secretion of these hormones is affected, among others, by rate of glucose synthesis (Roche *et al.*, 2008) and by variation of blood volatile fatty acids, mainly propionate (Bines and Hart, 1984), which its flux to the liver is increased after feeding in early and mid-lactation cows (Benson *et al*., 2002). The increase in the hepatic ATP:ADP ratio post-feeding in this treatment and the negative correlation between ATP:ADP ratio and acetyl-CoA may be indicating hepatic oxidation of fuels in the TCA cycle. Taken together, the results from TMR treatment are consistent with the hepatic oxidation theory, which suggests that the satiety signal generated from the liver to the brain during a feeding event seems to be more related to a balance between ATP production and utilization than ATP concentration *per se* (Allen and Piantoni, 2013; Allen, 2014). Nevertheless, the positive correlation observed between post-feeding ATP concentration and the duration of first non-feeding event may also reflect an effect of hepatic ATP concentration in the maintenance of satiety state. On the other hand, the negative correlation between pre-feeding ATP:ADP ratio and DM intake in the first feeding event may indicate that low pre-feeding energy load of the liver could also be a stimulatory signal for intake (Friedman, 1997). Furthermore, the negative correlation between DM intake in the first feeding event and the decrease in plasma glucose could be associated with individual cow responses to insulin (Bradford and Allen, 2007).

In PAS treatment, unlike TMR, the lack of variation in plasma glucose between pre- and post-feeding sampling was consistent with the unchanged circulating insulin and insulin:glucagon ratio. These results were in agreement with data reported by Sheahan *et al*. (2013b) who observed that glucose and insulin concentrations remained constant during the first 80 min (approx.) after pasture was offered in the a.m. grazing bout. In addition, we observed a lower rumen volatile fatty acids pool in PAS than TMR treatment and a delay in reaching the highest volatile fatty acids pool

after the first feeding event in PAS than TMR cows (data not shown, estimated by run CTR model, Chilibroste *et al*., 2008). These could explain the lack of variation between pre- and post-feeding liver ATP:ADP ratio in PAS cows. Interestingly, the negative correlations observed between intake rate and post-feeding liver ATP:ADP ratio and between intake rate and the increase in serum BHB concentration, as well as the positive correlation between the increase in serum BHB concentration and post-feeding liver ATP:ADP ratio, highlight the importance of intake rate in the availability of nutrients under grazing condition (Ulyatt *et al*., 1986). Collectively, the results from PAS treatment, do not support the hepatic oxidation theory as to be a primary signal controlling the first feeding event cessation under grazing high digestible pastures.

2.7. CONCLUSION

The type of diet affects the DM intake feeding strategy and the availability of nutrients during a feeding event. Hepatic and metabolic signals known to be associated with intake regulation in TMR fed dairy cows appear not to have a primary role in the cessation of the first feeding event in grazing dairy cows in this experiment. Further research relating to intake rate, flux of nutrients to the liver during meals and its response in endocrine-metabolic signals and hepatic metabolism is needed to better understand the metabolic control of short feed intake in grazing dairy cows.

2.8. ACKNOWLEDGEMENT

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2.9. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

2.10. REFERENCES

Allen MS (2000) Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science* **83**, 1598–1624.

Allen MS, Bradford BJ, Oba M (2009) Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. *Journal of Animal Science*, **87**(10), 3317–3334.

Allen MS, Piantoni P (2013) Metabolic control of feed intake. Implications for metabolic disease of fresh cows. *Veterinary Clinics of North America - Food Animal Practice* **29**, 279–280.

Allen MS (2014) Drives and limits to feed intake in ruminants. *Animal Production Science* **54**, 1513–1524.

Ametaj BN, Emmanuel DGV, Zebeli Q, Dunn SM (2009) Feeding high proportions of barley grain in a total mixed ration perturbs diurnal patterns of plasma metabolites in lactating dairy cows. *Journal of Dairy Science* **92**, 1084–1091.

AOAC (2000) ´Official methods of analysis.´ 17th edn. (Association of Official Analytical Chemists: Gaithersburg, MD)

Astessiano AL, Meikle A, Fajardo M, Gil J, Mattiauda DA, Chilibroste P, Carriquiry M (2015) Metabolic and endocrine profiles and hepatic gene expression of Holstein cows fed total mixed ration or pasture with different grazing strategies during early lactation. *Acta Veterinaria Scandinavica* **57**, 1–12.

Bargo F, Muller LD, Delahoy JE, Cassidy TW (2002) Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science* **85**, 2948–2963.

Benson JA, Reynolds CK, Aikman PC, Lupoli B, Beever DE (2002) Effects of abomasal vegetable oil infusion on splanchnic nutrient metabolism in lactating dairy cows. *Journal of Dairy Science* **85**, 1804–1814.

Bines JA, Hart IC, Morant SV (1983) Endocrine control of energy metabolism in the cow: diurnal variations in the concentrations of hormones and metabolites in the

blood plasma of beef and dairy cows. *Hormone and Metabolic Research* **15**, 330–334.

Bines JA, Hart IC (1984) Intraruminal infusion of VFA mixtures in cattle. *Canadian Journal of Animal Science* **64**, 304–305.

Bradford BJ, Allen MS (2007) Depression in feed intake by a highly fermentable diet is related to plasma insulin concentration and insulin response to glucose infusion. *Journal of Dairy Science* **90**, 3838–3845.

Cabrera Estrada JI, Delagarde R, Faverdin P, Peyraud JL (2004). Dry matter intake and eating rate of grass by dairy cows is restricted by internal, but not external water. *Animal Feed Science and Technology* **114**, 59–74.

Carriquiry M, Weber WJ, Fahrenkrug SC, Crooker BA (2009) Hepatic gene expression in multiparous Holstein cows treated with bovine somatotropin and fed n-3 fatty acids in early lactation. *Journal of Dairy Science* **92**, 4889–4900.

Chilibroste P, Tamminga S, Boer H (1997) Effects of length of grazing session, rumen fill and starvation time before grazing on dry-matter intake, ingestive behaviour and dry-matter rumen pool sizes of grazing lactating dairy cows. *Grass and Forage Science* **52**, 249–257.

Chilibroste P, Tamminga S, Van Bruchem J, Van der Togt PL (1998) Effect of allowed grazing time, inert rumen bulk and length of starvation before grazing, on the weight, composition and fermentative end-products of the rumen contents of lactating dairy cows. *Grass and Forage Science* **53**, 146–156.

Chilibroste P (1999) ´Grazing time: the missing link? A study on the plant-animal interface by integration of experimental and modeling approaches.´ PhD thesis, Wageningen University, The Netherlands.

Chilibroste P, Soca, P, Mattiauda DA, Bentancur O, Robinson PH (2007) Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. *Australian Journal of Experimental Agriculture* **47**, 1075–1084.

Chilibroste P, Dijkstra J, Robinson PH, Tamminga S (2008) A simulation model "CTR Dairy" to predict the supply of nutrients in dairy cows managed under discontinuous feeding patterns. *Animal Feed Science and Technology* **143**, 148–173.

Chilibroste P, Mattiauda DA, Bentancur O, Soca P, Meikle A (2012) Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. *Animal Feed Science and Technology* **173**, 201–209.

Dairy One Interactive Feed Composition Libraries. Available: https://dairyone.com/analytical-services/feed-and-forage/feed-composition-library/interactive-feed-composition-library/.

Derno M, Nürnberg G, Schön P, Schwarm A, Röntgen M, Hammon HM, Metges CC, Bruckmaier RM, Kuhla B (2013) Short-term feed intake is regulated by macronutrient oxidation in lactating Holstein cows. *Journal of Dairy Science* **96**, 971–980.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G (1989) A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* **72**, 68–78.

Faverdin P (1999) The effect of nutrients on feed intake in ruminants. *Proceedings of the Nutrition Society* **58**, 523–531.

Forbes JM (1995) ´Voluntary food intake and diet selection in farm animals.´ (CAB International, Wallingford, UK)

Friedman MI (1997) An energy sensor for control of energy intake. *Proceedings of Nutritional Society* **56**, 41–50.

Gibb MJ, Huckle CA, Nuthall R (1998) Effect of time of day on grazing behaviour by lactating dairy cows. *Science* **53**, 41–46.

Gibb MJ, Huckle CA, Nuthall R, Rook AJ (1999) The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. *Applied Animal Behaviour Science* **63**, 269–287.

Gill M, Rommey D (1994) The relationships between the control of meal size and the control of daily intake in ruminants. *Livestock Production Science* **39**, 13–18.

Gregorini P, Tamminga S, Gunter SA (2006) Review: Behavior and daily grazing patterns of cattle. *Professional Animal Scientist* **22**, 201–209.

Gregorini P, Gunter SA, Masino CA, Beck PA (2007) Effect of ruminal fill on short-term intake rate and grazing dynamics of beef heifers. *Grass and Forage Science* **62**, 346–354.

Haydock KP, Shaw NH (1975) The comparative yield method for estimating dry matter yield of pasture. *Australian Journal of Experimental Agricultural and Animal Husbandry* **15**, 663–670.

Hills JL, Wales WJ, Dunshea FR, Garcia SC, Roche JR (2015) Invited review: An evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. *Journal of Dairy Science* **98**, 1363–1401.

Iqbal S, Zebeli Q, Mazzolari A, Dunn SM, Ametaj BN (2012) Barley grain-based diet treated with lactic acid and heat modulated plasma metabolites and acute phase response in dairy cows. *Journal of Animal Science* **90**, 3143–3152.

Jones DIH, Moseley G (1993) Laboratory methods for estimating nutritive quality. In ´Sward Measurement Handbook´. 2nd edn. (Eds A Davies, RD Baker, SA Grant, AS Laidlaw) pp. 265–283. (British Grassland Society: Reading)

Kennedy E, McEvoy M, Murphy JP, O'Donovan M (2009) Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. *Journal of Dairy Science* **92**, 168–176.

King MTM, Crossley RE, DeVries TJ (2016) Impact of timing of feed delivery on the behavior and productivity of dairy cows. *Journal of Dairy Science* **99**, 1471–1482.

Kolver ES, Muller LD (1998) Performance and nutrient intake of high producing holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science* **81**, 1403–1411.

Lafontan M, Sengenes C, Moro C, Galitzky J, Berlan M (2009) Natriuretic peptides and other lipolytic peptides involved in the control of lipid mobilization. In ´Peptides in Energy Balance and Obesity´. (Ed. G Frühbeck) pp. 133–161. (CAB Int.: Oxfordshire)

Mattiauda DA, Tamminga S, Gibb MJ, Soca P, Bentancur O, Chilibroste P (2013) Restricting access time at pasture and time of grazing allocation for Holstein

dairy cows: Ingestive behaviour, dry matter intake and milk production. *Livestock Science* **152**, 53–62.

Mendoza A, Cajarville C, Repetto JL (2017) Behaviour of cows fed a total mixed ration with different access time to fresh forage. *New Zealand Journal of Agricultural Research* **61**, 102–108.

Miller-Cushon EK, DeVries TJ (2017) Short communication: Associations between feed push-up frequency, feeding and lying behavior, and milk yield and composition of dairy cows. *Journal of Dairy Science* **100**, 2213–2218.

Moallem U, Folman Y, Sklan D (2000) Effects of somatotropin and dietary calcium soaps of fatty acids in early lactation on milk production, dry matter intake, and energy balance of high-yielding dairy cows. *Journal of Dairy Science* **83**, 2085–2094.

Nikkhah A (2014) Review: Ruminant feed intake regulation evolution: Chronophysiological rhythms perspectives. *Biological Rhythm Research* **45**, 563–577.

NRC (2001) ´Nutrient requirements of dairy cattle.´ (National Academies Press: Washington, DC)

Oba M, Allen MS (1999) Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science* **82**, 589–596.

Oba M, Allen MS (2003) Extent of hypophagia caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. *Journal of Nutrition* **133**, 1105–1112.

Parker WJ, McCutcheon SN, Carr DH (1989) Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. *New Zealand Journal of Agricultural Research* **32**, 537–546.

Parsons AJ, Thornley JHM, Newman J, Penning PD (1994) A mechanistic model of some physical determinants of intake rate and diet selection in a two-species temperate grassland sward. *Functional Ecology* **8**, 187.

Patterson DM, McGilloway DA, Cushnahan A, Mayne CS, Laidlaw AS (1998) Effect of duration of fasting period on short-term intake rates of lactating dairy cows. *Animal Science* **66**, 299–305.

Penning PD, Hooper GE (1985) An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. *Grass and Forage Science* **40**, 79–84.

Peyraud JL (1998) Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for investigating intake and ingestive behaviour by farm animals. In ´Proceedings of the 9th European intake workshop´. (Eds AMC Davies, R Giangiacomo) pp. 39–48. (IGER: North Wyke).

Phillips C (2002) Nutritional Behavior. In ´Cattle behavior and Welfare´. 2nd edn. (Ed. C Phillips) pp. 123–151. (Blackwell Science Ltd.: Cornwall)

Piantoni P, Ylioja CM, Allen MS (2015) Feed intake is related to changes in plasma nonesterified fatty acid concentration and hepatic acetyl CoA content following feeding in lactating dairy cows. *Journal of Dairy Science* **98**, 6839–6847.

Ribeiro Filho H, Peyraud JL, Delagarde R (2012) Foraging behavior and ruminal fermentation of dairy cows grazing ryegrass pasture alone or with white clover. *Pesquisa Agropecuária Brasileira* **47**, 458–465.

Roca-Fernández AI, Ferris CP, González-Rodríguez A (2013) Short communication. Behavioural activities of two dairy cow genotypes (Holstein-Friesian *vs.* Jersey × Holstein-Friesian) in two milk production systems (grazing vs. confinement). *Spanish Journal of Agricultural Research* **11**, 120–126.

Roche JR, Blache D, Kay JK, Miller DR, Sheahan AJ, Miller DW (2008) Neuroendocrine and physiological regulation of intake with particular reference to domesticated ruminant animals. *Nutrition Research Reviews* **21**, 207–234.

Rossi R, Dorig S, Del Prete E, Scharrer E (2000) Suppression of feed intake after parenteral administration of D-beta-hydroxybutyrate in pygmy goats. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine* **47**, 9–16.

Sales J, Janssens G (2003) Acid-insoluble ash as a marker in digestibility studies: a review. *Journal of Animal and Feed Sciences*, **12**, 383–401.

Sheahan AJ, Boston RC, Roche JR (2013a) Diurnal patterns of grazing behavior and humoral factors in supplemented dairy cows. *Journal of Dairy Science* **96**, 3201–3210.

Sheahan AJ, Kay JK, Roche JR (2013b) Carbohydrate supplements and their effects on pasture dry matter intake, feeding behavior, and blood factors associated with intake regulation. *Journal of Dairy Science* **96**, 1–12.

Sheahan AJ (2014) ´Neuroendocrine regulation of dry matter intake in grazing dairy cows.´ PhD thesis, Lincoln University, New Zealand.

Stakelum G, Dillon P (2003) The effect of supplement type on the rumen fermentation pattern of cows fed fresh grass and the in sacco disappearance of grass in the rumen. *Irish Journal of Agricultural and Food Research*, **42**(2), 213–228.

Sutton JD, Hart IC, Broster WH, Elliott RJ, Schuller E (1986) Feeding frequency for lactating cows: effects on rumen fermentation and blood metabolites and hormones. *The British Journal of Nutrition* **56**, 181–192.

Taweel HZ, Tas BM, Dijkstra J, Tamminga S (2004) Intake Regulation and Grazing Behavior of Dairy Cows Under Continuous Stocking. *Journal of Dairy Science* **87**, 3417–3427.

Tejada de Hernandez I (1983) Análisis de forrajes, ensilajes y líquido ruminal. In ´Manual de laboratorio para análisis de ingredientes utilizados en la alimentación animal´. (Ed. Instituto de Investigaciones Pecuarias en Mexico) pp. 299–300. (SARH-INIP: Mexico)

Thorne P, Jago J, Kolver E, Roche J (2003) Diet and genotype affect feeding behaviour in Holstein-Friesian dairy cows during late lactation. *Proceedings of the New Zealand Society of Animal Production* **63**: 124–127.

Tóthi R, Zhang RH, Chilibroste P, Boer H, Tamminga S (2003) Effect of processed cereal grains as a supplement on grass intake, rumen pool sizes, ruminal kinetics and the performance of grazing lactating dairy cows. *Journal of Animal Feed and Science* **12**, 417–433.

Tyrrell HF, Reid JT (1965) Prediction of the energy value of cows' milk. *Journal of Dairy Science* **48**, 1215–1233.

Ulyatt MJ, Dellow DW, Jhon A, Reid CSW, Waghorn GC (1986) Contribution of chewing during eating and rumination to clearance of digesta from the ruminum-reticulum. In ´Control of Digestion and Metabolism in Ruminants´. (Eds LP Milligan, WL Grovum, A Dobson) pp. 498–515. (Prentice Hall, Inc., Englewood Cliffs)

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Waghorn GC (2002) Can forages match concentrate diets for dairy production? *Proceedings of the New Zealand Society of Animal Production* **62**, 261–266.

Wylie ARG, Woods S, Carson AF, McCoy M (2008) Periprandial changes in metabolite and metabolic hormone concentrations in high-genetic-merit dairy heifers and their relationship to energy balance in early lactation. *Journal of Dairy Science* **91**, 577–586.

Zarrin M, De Matteis L, Vernay MCMB, Wellnitz O, Van Dorland HA, Bruckmaier RM (2013) Long-term elevation of β-hydroxybutyrate in dairy cows through infusion: effects on feed intake, milk production, and metabolism. *Journal of Dairy Sci*ence **96**, 2960–2972.

3. ENDOCRINE-METABOLIC CONTROL OF FIRST DAILY GRAZING MEAL OF LACTATING DAIRY COWS

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Short title: First grazing meal control

3.1. HIGHLIGHTS

- We measured the 1st a.m. meal and the daily herbage DMI of cows in early lactation.
- We studied metabolic-endocrine state as signals of DMI control of the 1st meal.
- The 1st meal comprises 40% of daily herbage DMI in 8 h access time to pasture.
- The BHB concentration would seem to play a role in controlling the 1st meal.

3.2. ABSTRACT

Nine multiparous (**MUL**) and 9 primiparous (**PRI**) Holstein cows in their eighth week of lactation were allowed to access together to a vegetative oat pasture for 8 h daily. Each cow received approximately 6 kg DM of a supplementary mixed ration (**MR**, 55:45 forage to concentrate ratio) after pm milking. Cows were used in a randomized complete block design to assess their metabolic state (glucose, non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHB**) and insulin concentration) in response to the first daily grazing meal in a short-term experiment (15 d adaptation, 5 d measurement). During the measurement period, milk production was daily recorded and the DM intake/cow (herbage and MR) was measured. The temporal pattern of grazing activity was recorded by visual observation at 5-min intervals on days 16 to 18. Herbage intake, grazing time and number of bites during the first grazing meal after release to pasture were measured on days 19 and 20, from which mean intake rate (g DM/min) and bite mass (g) were calculated. Blood samples were collected pre- and post- grazing meal, for determination of metabolites and insulin concentrations. Although the daily mean meal duration and bite and intake rates did not differ significantly between parities, MUL cows grazed for 20 minutes longer (P=0.009) and achieved a greater daily herbage intake, total intake and estimated NE_L intake (P<0.05) compared with the PRI cows, contributing to significantly higher milk yield. Both PRI and MUL cows achieved approximately

40% of their daily herbage intake during their first grazing meal of the day. During that meal, MUL cows consumed 1 kg DM, and 1.67 Mcal of estimated NE_L more ($P < 0.05$) than PRI cows, which was achieved by their significantly greater bite mass and intake rate ($P = 0.013$ and $P = 0.014$, respectively). Blood glucose and NEFA concentrations decreased ($P < 0.050$) whereas BHB increased ($P < 0.001$) from the pre- to post-grazing meal in both parities. However, pre-grazing NEFA concentration and post-grazing BHB concentration were greater ($P = 0.001$) for MUL than PRI cows, whereas post-grazing glucose concentration was greater ($P = 0.035$) for PRI than MUL cows. The results indicate that metabolites such as BHB and/or NEFA appear to be involved in herbage DM intake control of first grazing meal differentially for MUL and PRI cows. More studies are needed to understand the complex interaction of ruminal, metabolic and hormonal mechanisms involved in grazing intake control of dairy cows.
Keywords: grazing meal, parity, oat pasture

3.3. INTRODUCTION

In pasture-based systems, cattle divide their grazing time into a series of discrete grazing meals separated by non-grazing intervals occupied by other activities (e.g. rumination, resting). Thus, total dry matter (DM) intake of herbage is determined by the number of grazing meals and the amount of herbage consumed in each individual meal. Signals involved in the control of a grazing meal will influence the regulation of daily herbage intake. Where cattle have continuous access to pasture, three or possibly four major grazing sessions may be identified, with that occurring at the end of the day usually being of the longest duration (Gibb, 2007). Removing dairy cows from pasture for milking imposes an enforced fast from grazing, particularly where distances from the paddock to the milking parlor are long or milking of large herds delay return to the field, and may disrupt the natural pattern of grazing behavioural in the subsequent meals (Chilibroste *et al*., 2015).

In this context, the signals that mediate the initiation and cessation of a grazing meal become even more complex. The main hypotheses regarding grazing meal control have focused on competition between behavioural requirements (grazing vs. rumination and resting), on physical and chemical signals generated at rumen level and on hunger-related hormones and metabolites concentrations (Chilibroste *et a*l., 2015). However, some results have showed that rumen fill effect and rumen fermentation end products may not play a major role in the initiation and/or termination of a grazing meal (Taweel *et al*., 2004), and the effect of hormones and metabolites concentrations affecting the regulation of a grazing meal is not yet totally understood (Gregorini *et al*., 2009; Sheahan *et al*., 2013). Therefore, the internal state of cows, which plays an important role in the animal's perception of the pasture as a feed resource and its utilization by the act of grazing (Gregorini *et al*., 2009), is an important area of study required to further our understanding of the signals involved in the control of grazing meal initiation and duration, and thus herbage intake.

The effect of parity also plays a role in feed intake regulation. Cows in their first lactation consume less food and yield less milk than multiparous cows (e.g. Peyraud *et al*., 1996; Maekawa *et al*., 2002; Bowman *et al*., 2003) together with greater

demands for nutrients for their own growth and increased requirements for their first lactation (Cissé *et al*., 1991). However, few studies have compared the differences in grazing behavioural between lactating multiparous and primiparous cows grazing together (Peyraud *et al*., 1996; Phillips and Rind, 2001). These authors reported that when grazing together primiparous cows had daily grazing times similar to those of multiparous cows, but achieved lower daily herbage intakes. The effect of parity on short-term endocrine-metabolic control of feed intake in peak lactation grazing dairy cows has received little attention. We hypothesized that short-term herbage intake is linked to the endocrine-metabolic state of dairy cows, which may also be affected by parity. The aim of this experiment was to study the dairy cows' internal state as a source of signals regulating short-term intake, assessing the metabolic state (glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and insulin) of multiparous and primiparous, before and after the first daily grazing meal, and relate them to behavioural pattern at pasture and to daily herbage intake.

3.4. MATERIALS AND METHODS

The experiment was carried out at the Experimental Research Station "Dr. M.A. Cassinoni" (EEMAC) of the Facultad de Agronomía (Paysandú, Uruguay, 32º22'52" S, 58º03'10.25" W) during May of 2016, in the autumn season. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (UdelaR, Montevideo, Uruguay), under application number 021130-002616-14.

3.4.1. Animals and experimental design

Nine multiparous (MUL) and 9 primiparous (PRI) Holstein cows were used in a randomized block design for 20 days, allowing 15 days for adaptation to experimental management and pasture, followed by 5 days for experimental determinations. At the start of the experiment, MUL cows (8 in their 2nd lactation and 1 in its 3rd lactation) averaged (± SD) 72 ± 2.3 days in milk (DIM), 540 ± 8.8 kg of live weight (LW), 2.6 ± 0.06 body condition score (BCS), 28.5 ± 0.53 kg of milk yield and 4 years old, while PRI cows were in 74.9 ± 2.3 DIM, 501 ± 8.8 kg of LW, 2.8 ± 0.06 BCS, 23.3 ± 0.53 kg of milk yield and 3 years old. Cows were selected

from the autumn calving herd, blocked by DIM, LW, BCS, number of lactations and milk yield, comprising 9 blocks of 1 MUL and 1 PRI.

3.4.2. Pasture and animal management

The oat pasture (*Avena byzantina*; Table 1) provided for grazing was sown in March 2016 with 120 kg/ha of seed and fertilized with 46 kg of N in April. During the experimental period, herbage DM mass (kg/ha) to ground level was measured weekly using a double sampling technique. At five locations chosen subjectively to represent a range of pasture heights, sward heights were measured to the nearest 0.5 cm, using a rising plate meter (RPM, Ashgrove Co., Palmerston North, NZ). Herbage was then cut at ground level from within 30 x 30 cm frames placed at the sites measured using the RPM, and dried to constant weight (Mattiauda *et al*., 2013). A local regression was then calculated relating herbage DM mass to RPM height, and applied to the mean RPM height of the pasture measured at 50 random locations.

Cows were milked twice daily, at 04:00 and 17:00 h. After morning milking, they were kept in a pen with access to water until 8:30 h, when MUL and PRI cows were released together onto their daily strip of pasture calculated to provide an herbage allowance of 30 kg of DM/cow/day based on the mean herbage DM measured, as above. No pastures were re-grazed during the experiment. After p.m. milking cows were individually fed a supplementary mixed ration (MR, 55:45 forage to concentrate ratio, as-dry basis) formulated to meet their maintenance energy requirements (Table 1) and remained together in a pen with access to water until a.m. milking.

Table 1. Supplementary mixed ration (MR) ingredients, the chemical composition of MR and herbage samples[1], and their estimated content of net energy available for lactation (NE_L).

Item	MR	Herbage [1]
Ingredients (g/kg)		
Corn silage	503	
Lucerne hay	41	
Corn grain	146	
Barley grain	141	
Soybean meal	146	
Salt (NaCl)	5	
Compound salt	18	
Analyzed Composition		
DM (g/kg)	524	130
OM (g/kg DM)	932	882
NDF (g/kg DM)	357	454
NDIN(g/kg)	20	25
ADF (g/kg DM)	193	277
CP (g/kg/DM)	142	200
EE (g/kg DM)	25	36
NE_L (MJ/kg DM)[2]	7.53	6.68

[1] Sward grazing horizon was representatively sampled using electric hand-held shears before the first grazing event, from day 7 to 11 during the intake measurement period.

[2] Estimated according to NRC (2001)

3.4.3. Animal and sample analyses

3.4.3.1. Milk production, body weight and body condition score

During the 5 days of measurements, individual milk yields (kg) were recorded at each milking (Waikato MKV® Milk Meter, Hamilton, NZ). Milk fat, protein, and lactose concentrations were determined from one successive a.m. and p.m. milk sample taken on the third day (MilkoScan® Foss FT2, Hillerød, Denmark). Fat corrected milk yield **(**4%, FCM**)** was calculated using the equation of Tyrrell and Reid (1965). The LW and BCS (scale of 1-5, Edmonson *et al*., 1989) were recorded once at the beginning of the experiment.

3.4.3.2. Total dry matter and energy intake estimation

The total DM intake was estimated during the experimental period using a double marker analyses technique, using Cr2O3 as an external indigestible fecal marker to estimate fecal production (Peyraud, 1998) in conjunction with acid insoluble ashes (AIA) in feces and diet as an internal marker (Sales and Janssens, 2003). All cows were dosed twice daily, after each milking, for 12 consecutive days (7 days for adaptation and regulation of marker excretion flow and 5 days for fecal collection) with a bolus of paper containing 7.5 g of Cr_2O_3 (60% purity). After dosing, animals were observed to ensure that there was no regurgitation. Fecal grab samples were collected from the rectum of each cow twice daily after milking for subsequent determination of Cr_2O_3 (Parker *et al*., 1989). During the intake measurement period, sward grazing horizon was representatively sampled before first grazing meal (using electric garden hand shears; combined shears GSL35 Black&Decker), and, representative samples of the MR offered at each feeder were collected, as were samples of any refusals remaining after 45 min of voluntary cessation of MR intake. All samples were frozen at -20°C until dried at 60 °C for 48 h, and were bulked to obtain one sample/cow. The DM, crude protein (CP), ether extract (EE), ash and acid insoluble ash (AIA) content were determined according to AOAC (2000) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) as described by Van Soest *et al*. (1991) using alfa amylase and expressed subtracting ashes. The NDIN and ADIN also were determined (Licitra *et al*., 1996).

Herbage DM intake and total DM intake were calculated as:

Herbage DM intake (kg DM/cow/day) = $\{(F * [AIA_F]) - (MR * [AIA_{MR}])\} / [AIA_{HERBAGE}]$

Total DM intake (kg DM/cow/day) = MR intake + Herbage DM intake

where F = daily faecal DM production (Cr ingested (mg/day) / Cr in faeces (mg/kg DM)); MR = mixed ration (kg DM); $[AIA_F]$, $[AIA_{MR}]$, and $[AIA_{HERBAGE}]$ = concentration of insoluble ashes (%) in faeces, MR and herbage, respectively.

Daily energy balance (MJ of NEL/d) was estimated as the theoretical energy intake (NE_L intake, MJ/d) minus energy requirements for milk production (NE_L milk) and minus maintenance requirements (adjusted by walking and grazing activity and expressed in NE_L units).

The theoretical NE_L intake was calculated adding the MR intake and oat herbage intake multiplied by their respective NE_L concentration (derived from chemical composition of samples collected according to NRC 2001).

The energy requirements were calculated according to NRC (2001). Briefly, energy requirements for maintenance, adjusted by walking and grazing activity, was calculated as NE_L (Mcal) = ((0.08 × LW0.75 ×) + (distance × trips × (0.00045 × LW)) + (0.0012 × LW × (pasture/diet ratio)) and converted to MJ. The requirement for lactation was calculated as NE_L = Milk Yield × ((0.0929 × Fat %) + (0.0547 × Crude Protein %) + (0.0395 × Lactose %)), using the mean composition data derived from analyses of the milk samples collected and converted to MJ. Extra energy expenditures by first-lactation cows (PRI) was calculated considering a mean target shrunk live weight (SLW= 0.96 x LW) of 540 kg (that was de LW of MUL), according to NRC (2001) and converted to a MJ. Requirements for pregnancy was not considered as established in NRC 2001 (requirement starts at 190 days of gestation).

3.4.3.3. Temporal pattern of grazing

Through the first three days of the measurement period, cows were observed every 5-min whist at pasture and grazing, ruminating or resting activities were recorded. Grazing was defined as the combination of activities involved in consuming herbage (biting, chewing, ingestion, searching, etc.). Ruminating was defined as chewing the cud in a lying or standing position. Resting was defined as any activity other than grazing or ruminating. If the activity recorded was grazing, the number of bites made in one minute were counted by the observers to determine the bite rate (Chilibroste *et al*. 2012). To determinate the activity budget pattern, the probability of finding cows in each activity was calculated as the sum of each recorded activity divided by the

total observations. The total time (min) spent on each activity was calculated according to the proportion of time at pasture (approx. 8 h) in that activity. A grazing meal was defined when the activity of grazing was maintained for at less 2 consecutives records. The duration of the first non-grazing meal was defined as the time between the cessation of the first grazing meal and the start of a new grazing meal. Intake rate was calculated by dividing daily herbage intake by total grazing time.

3.4.3.4. Dry matter intake and intake rate of the first grazing meal

During the adaptation period, the cows were familiarized with the harnesses, excreta collection bags, procedures and the presence of observers. Herbage dry matter intake (kg DM), intake rate (gDM/min), bite rate (bites/min) and bite mass (g) of the first grazing meal were calculated from measurements made during the last two days of measurements (after morning milking) for each cow. Before grazing, cows were fitted with excreta collection bags to avoid excreta losses. Cows were weighed using a precision balance (accurate to 50 g) in a location protected from wind, 100 meters away from the paddock. Three measurements per second were recorded using a complement of Windows (Hyper Terminal private edition v.7.06 electronic Download) until a measurement was repeated at least 10 times. The LW was calculated as the mode (the most frequent value) of the recorded data. Herbage intake was calculated by weighing each cow before grazing and after voluntary cessation of the first grazing meal. Weight changes were corrected for the rate of insensible weight loss measured over approximately 1 hour before grazing, adapted from the procedure described by Penning and Hooper (1985). The weight of herbage DM consumed was derived from the calculated fresh weight of herbage consumed and the DM content determined from representative herbage samples. The number of bites per minute was counted every two minutes during the first grazing meal, and total number of bites was calculated as the product of bite rate and the duration (mins) of the meal. Mean bite mass was estimated by dividing herbage intake by the estimated total number of bites. Mean bite rate was calculated by dividing total bites by duration of first grazing meal, and mean intake rate by dividing herbage DM

intake by duration of the first grazing meal. The incisor arcade breadth was measured from a dental arcade impression of each cow (Illius and Gordon, 1987). The DM content, chemical composition and NE_L were estimated from hand clipped samples representative of the herbage selected by the cows during the initial grazing meal (Jones and Moseley, 1993). The DM during the first grazing meal was calculated as a percentage of the total daily herbage intake. Similarly, the estimated NE_L intake during the first grazing meal was calculated as a percentage of the estimated total daily NE_L intake derived herbage.

3.4.3.5. Blood sampling and analyses

Blood samples for determination of metabolite and insulin concentrations were collected prior to the first daily grazing meal (pre-grazing meal), following a fast of at least 12 h duration, and immediately after each cow had voluntarily ceased grazing (post-grazing meal). Pre-grazing samples of the first daily grazing meal were collected the day before the measurement period. Blood samples were collected by venipuncture of the coccygeal vein into two vacuum tubes, one for serum (BD Vacutainer® REF 367820), and the other for plasma containing Sodium Fluoride and Potassium Oxalate (BD Vacutainer® REF 367922) as a glycolytic inhibitor. Both tubes were centrifuged (2000 x g for 15 min at 4 □C) within 2 h after collection and plasma and serum were stored at −20 □C until analyzed.

Each metabolite and insulin was determined in a single assay. Plasma glucose was determined by colorimetric assays on Vitalab Selectra II autoanalyzer (Vital Scientific, Dieren, The Netherlands) using a commercial kit (BioSystems S.A. Costa Brava, 30. 08030 Barcelona, Spain). Serum NEFA and β-hydroxybutyrate (BHB) concentrations were analyzed by spectrophotometry using commercial kits: HR Series NEFA-HR (2), Wako and BHB A25-Biosystem Cod: 12525, respectively. Intra-assay CV for all determinations was ≤ 10%. Serum concentrations of insulin were measured using an immunoradiometric assay (IRMA) with a commercial kit (DIA source INS-IRMA Kit) previously used in cattle (Astessiano *et al.*, 2015). The assay detection limit was 1.518 μIU/ml, and intra-assay CV for control 1 (23.4 μIU/ml) and 2 (76.3 μIU/ml) were 6.2 and 2.2 %, respectively.

3.4.4. Statistical analyses

All statistical analyses were performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), except otherwise indicated. Residuals were tested for normality using the Shapiro-Wilk test (PROC UNIVARIATE statement).

Total DM intake, components of energy balance, daily milk and constituents yields, milk composition, and first grazing meal variables were analyzed by the mean of each individual variable using ANOVA. The model included the effect of parity and block as fixed and random effect, respectively.

Model: $Yij = \mu + Pi + Bj + Eij$

where μ = mean; Pi = parity (i = 1 to 2); Bj = block (j = 1 to 7); and Eij = residual error term.

To determine the probability of the different activities (grazing, ruminating and resting) a logistic regression model was performed using the PROC GLIMMIX procedure with a binomial response distribution. The model included the fixed effect of parity, and, block and cow nested within day, were included as random effects.

Blood metabolite concentrations and insulin were analyzed with repeated measurements over time where the cow was the subject using the following model:

$$Yijl = \mu + Pi + Bj + Eij + Hl + (T*H)il + dijl$$

Where μ = mean; Pi = parity (i = 1 to 2); Bj = block (j = 1 to 7); Eij = residual error term; Hl = time of sampling (repeated measured); (PxH)il = parity x time of sampling; and dijl = residual error of the repeated measure. This model included parity as fixed effect, block and cow as a random effect and moment as the repeated effect. Autoregressive covariance structure of the first order (AR-1) was used for all the models.

The relationship between herbage DM intake at the first grazing meal and pre- and post- metabolic variables were analyzed by Pearson correlations using PROC CORR

(SAS Inst. Inc.). For all results, means were considered to differ when $P \leq 0.05$ of Tukey test, and trends were identified when $0.05 < P \leq 0.10$. Data are presented as least square means ± pooled standard errors.

3.5. RESULTS

3.5.1. Total dry matter intake and energy balance

The MUL cows consumed more total DM and herbage DM, providing a greater theoretical intake of NE_L than the PRI cows ($P < 0.05$, Table 2). The MUL cows produced 5.2 kg/d (22%) more milk than PRI cows ($P < 0.001$). After correction for fat content, MUL cows produced 28% more milk than PRI cows. Yields of fat, protein and lactose were also greater for MUL than PRI cows ($P \leq 0.001$) but MUL cows produced milk with similar lactose content, with a tendency to greater ($P = 0.090$) fat content and lower ($P = 0.053$) protein content than PRI cows. The calculated NE_L in milk was greater ($P < 0.001$) for MUL than PRI cows as well as the estimated requirements of maintenance, but the energy balance were similar between parities.

Table 2. Effect of parity on total daily intake of oat herbage and supplementary mixed ration, estimated energy balance, lactation performance and grazing behaviour recorded during 8-h access to pasture and during the first grazing meal of the day.[1]

	Parity[2]			
	MUL	PRI	SEM	*P*-value
Total daily feed DM intake (kg)	18.8	16.0	0.69	0.010
Oat herbage intake	12.5	10.3	0.62	0.021
Mixed ration intake	6.3	5.7	0.12	0.005
Estimated daily NE balance (MJ)[3]				
Total NE_L intake[4]	126.54	109.03	4.58	0.016
Maintenance and grazing activity	41.28	38.93	0.52	0.001
Lactation	91.39	72.58	1.75	<0.001
Growth[5]		1.38	0.31	
Balance	-6.15	-3.84	5.06	0.648
Mean daily milk yield (kg)				
Milk	28.5	23.3	0.53	<0.001
Fat corrected milk (4%)	29.2	22.7	0.55	<0.001
Fat	1.2	0.9	0.03	<0.001
Protein	0.9	0.8	0.02	0.001
Lactose	1.4	1.2	0.03	<0.001
Total milk solids	3.6	2.9	0.06	<0.001
Milk composition (% of FM[6])				
Fat	4.2	3.9	0.12	0.090
Protein	3.3	3.5	0.07	0.053
Lactose	5.1	5.0	0.04	0.254
Grazing behaviour during 8-h access to pasture[7]				
Intake rate (g DM/min)	38.7	33.5	2.43	0.163
Number of grazing meals	4.1	4.1	0.31	0.789
Mean grazing meal duration (min)	87.7	77.9	6.53	0.262
Mean bite rate (bites/min)	47.0	46.3	1.98	0.676
Total grazing time (min)	318.6	298.6	6.90	0.009
Total rumination time (min)	75.4	78.8	6.00	0.676
Resting time (min)	89.6	106.4	6.40	0.020
First non-grazing event duration (min)	56.4	67.2	5.83	0.198
Grazing behaviour during first grazing meal[8]				
Intake (kg DM)	5.0	4.0	0.32	0.044
Mean intake rate (gDM/min)	46.9	38.5	1.99	0.013
Mean bite mass (gDM)	0.90	0.72	0.05	0.014
Mean bite rate (bite/min)	52.4	53.6	1.58	0.571
Meal duration (min)	107.8	107.2	9.26	0.955
NE_L intake (MJ)[9]	34.09	27.07	2.20	0.040
Incisor arcade breath (cm)	9.21	8.05	0.05	<0.001
First grazing meal intake/herbage intake (%)	41.8	39.6	4.00	0.708
First grazing meal intake/total intake (%)	27.6	25.3	2.41	0.509
NE_L intake of first grazing meal/total NE_L intake (%)	27.9	24.9	2.42	0.407

[1] Least squares mean and SEM (n=18).

[2] Parity: multiparous (MUL) and primiparous (PRI) cows grazing in a single paddock.

[3]NE balance: estimated by total NE_L intake minus total net energy requirements for milk production (lactation) and maintenance and grazing activity, calculated according to NRC (2001).

[4]NE_L was calculated from adding MR intake and oat herbage intake multiplied by their respective NE_L concentration (NRC 2001).

[5]Grazing behavior was recorded every five minutes throughout the 8-h period at pasture on the first 3 days of the experimental measurement period.

[6]Bites rates per minute were counted every 2 minutes throughout the first grazing meal on the last 2 days of the experimental measurement period.

[7]NE_L intake of the first grazing meal was calculated from the first grazing meal intake multiplied by its NE_L concentration (NRC 2001)

3.5.2. Temporal pattern of grazing and first grazing meal

Results of temporal pattern of grazing and observations during the first grazing meal, are shown in Table 2. The probability of finding cows grazing during the 8-hour period at pasture was greater (P = 0.009) for MUL than PRI cows, equivalent to 20 min. The number of grazing meals and the average grazing meal duration did not differ between parities, nor did the mean bite rate or intake rate. In contrast, the probability of finding cows resting was greater (P = 0.020) in PRI cows, and no differences between treatments were detected in rumination time and the first non-grazing meal duration.

Both PRI and MUL cows consumed approximately 40% of their total daily herbage intake. The MUL cows had significantly greater bite masses than the PRI cows (P = 0.013), in accordance with their greater incisor arcade width, and higher short-term intake rates (P = 0.014). Although meal duration was similar, MUL cows were able to consume 1 kg DM and 1.67 Mcal of estimated NE_L more than PRI cows ($P < 0.05$) during this first grazing meal.

3.5.3. Pre- and post-grazing metabolic-endocrine variables

The pre-grazing serum NEFA concentrations were greater in MUL than PRI cows ($P = 0.001$) and decreased ($P < 0.001$) to similar levels in MUL and PRI cows by the end of the grazing meal (Figure 1 A). The BHB concentrations did not differ between parities before grazing, but had increased by the end of the grazing meal ($P < 0.001$), with post-grazing concentration being greater in MUL than in PRI cows ($P = 0.001$) (Figure 1 B). Before grazing, glucose concentration was not affected by parity, but had declined ($P < 0.05$) by the end of the grazing meal, when the mean concentration was lower in MUL than PRI cows ($P = 0.035$) (Figure 1 C). No significant differences in insulin concentrations were detected between MUL and PRI cows, nor between the pre- and post-grazing meal (Figure 1 D).

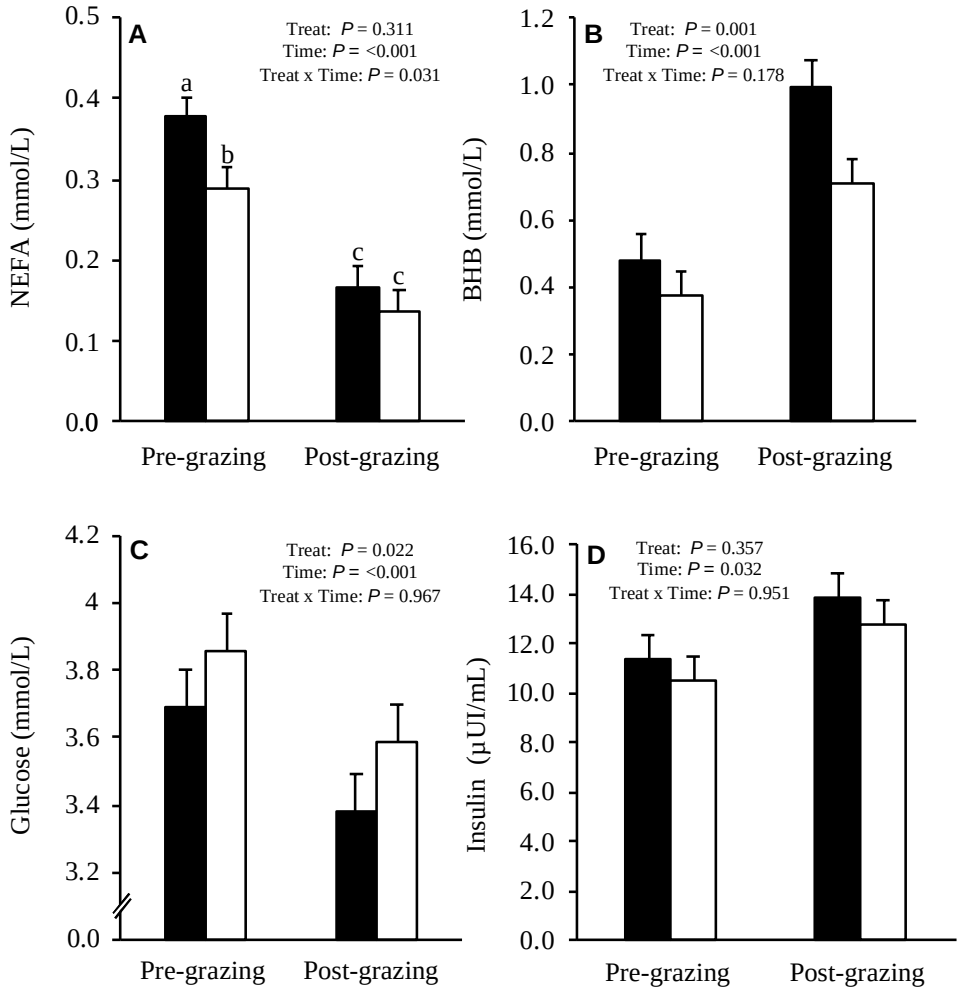

Figure 1. Blood concentrations of NEFA, BHB, glucose and insulin (A, B, C and D, respectively) immediately before and after the first grazing meal of the day in multiparous and primiparous cows. Treat: treatments (multiparous-solid column and primiparous-open column), Time: Time of sampling (pre- and post-grazing meal). Different letters between treatments indicates significant differences ($p<0.05$).

Regression analyses, using data from MUL and PRI cows together, showed a slight correlation between short-term intake rate and pre-grazing NEFA concentration ($r = 0.41$; $P = 0.100$). The same analyses showed a correlation between post-grazing BHB concentration and herbage DM intake achieved during the meal ($r = 0.63$; $P = 0.006$), and between post-grazing BHB concentration and mean intake rate during the meal

(r = 0.66; *P* = 0.004).

3.6. DISCUSSION

In Uruguay, most commercial dairy farms are from small to medium scale (DIEA, 2018), where management of multiparous and primiparous cows in a single herd is a common practice utilized by pastoral dairy farmers (Pereira *et al*., 2017). Therefore, the experiment was designed to study changes in blood metabolite and insulin levels as regulatory signals of the first daily grazing meal as affected by parity when grazing together, and to relate them to the behaviour during this meal, and during the usual 8-hour period of access to pasture afforded to the cows. Differences observed between MUL and PRI cows in the endocrine-metabolic profile during the first daily grazing meal and in their grazing behaviour, confirmed the hypothesis that short-term herbage intake is linked to endocrine-metabolic state of dairy cows, and this state is affected by parity.

The greater milk yields achieved by multiparous compared with primiparous cows are frequently explained by greater daily intakes attained by multiparous (Dado and Allen, 1994; Beauchemin and Rode, 1994; Peyraud *et al*., 1996; Nikkhah, 2012). In this experiment, the greater milk and milk solids yields by the MUL compared with the PRI cows (22% and 24% higher yields, respectively) appear to have been achieved largely due to the greater daily intakes of herbage DM by the MUL cows respect to PRI (21%).

The temporal pattern of grazing in the current study was similar to that reported elsewhere for dairy cows when provided a restricted daily grazing session and supplement ration (Pérez-Ramírez *et al*., 2008; Sheahan *et al*., 2013; Gregorini *et al*., 2017). However, in contrast to previous studies of multiparous and primiparous cows grazing together, where differences in grazing time between parities were not found (Peyraud *et al*., 1996; Phillips and Rind 2001), in the present study MUL cows grazed for 20 minutes longer than PRI cows during the course of their 8 hours at pasture. The limited pasture access time (8 h) following a long fasting period, as imposed in the present experiment, compared with these previous studies (>16 h

access) may explain the proportionately greater differences in grazing time when grazing is constrained by such management. This longer grazing time attained by MUL cows, together with a non-significant, but 13.6% greater mean herbage DM intake rate compared with the PRI cows, enabled MUL cows to consume more herbage DM daily. Approximately 12% greater intake rates in multiparous compared with primiparous cows have also been reported by Peyraud *et al*. (1996), confirming our finding in this respect.

Daily herbage DM intake by the MUL cows was significantly greater than that by the PRI cows. However, the similar ruminating times by MUL and PRI cows, as observed during the 8-hour period at pasture, should not be interpreted as representative of ruminating activity over 24 hours, or indicative of ruminating efficiency. It should be noted that the relatively short ruminating times measured during the 8-hour recording period were undoubtedly constrained by grazing activity being confined to this period.

3.6.1. Control of first grazing meal intake

As was reviewed by Chilibroste *et al*. (2007), the fasting imposed during evening and night may had affected total grazing time, especially by affecting the first grazing meal, which is the most sensitive to changes in input of nutrients. In response to this, different signals generated in the first grazing meal may have affected subsequent grazing meal durations or the intervals between them, constraining total feed intake.

As the mean bite rates during the first grazing meal were similar for MUL and PRI cows, the greater intake rate achieved by the MUL cows can be explained by their greater bite mass, attributable to their broader incisor arcade (Gregorini *et al*., 2009). The greater bite mass taken by the MUL compared with the PRI cows, following their overnight and early morning fast, may have been stimulated by the physiological requirement to support the inherently greater daily milk production. Nevertheless, PRI cows were able to consume 39% of their calculated daily herbage intake during their first grazing meal, comparable with 40% for the MUL cows.

There was no indication from our results that the greater rumen fill achieved by the end of their first grazing meal in the MUL compared with PRI cows had any effect on subsequent grazing behavior.

The higher NEFA concentration observed in MUL compared with the PRI cows prior to grazing is probably indicative of the greater circulation of this metabolite necessary to support the greater level of milk production (Weber *et al*., 2013) of MUL cows as well as the tendency to a greater fat percentage. In addition, the prolonged fasting imposed during night in the current experiment probably reduced the contribution of consumed nutrients for milk production stimulating a lipolytic response with the consequent higher level of NEFA concentration (Chelikani *et al*., 2004) in MUL than in PRI cows in pre-grazing.

The changes in NEFA and glucose concentrations, and, the small although statistically non-significant, change in insulin concentration, over the first grazing meal are similar to changes observed by Sheahan *et al*. (2013) in multiparous dairy cows following grazing. The decrease in NEFA concentration and increase in BHB by the end of the grazing meal is commensurate with a change in the tissue metabolism, from a state of catabolism to anabolism (Lafontan *et al*., 2009). In addition, the slightly higher pre-grazing BHB concentration in MUL cows compared with PRI cows is consistent with the higher NEFA concentration shown at that time, reflecting the higher metabolic load due to their greater milk yields. The greater disparity between BHB concentrations in MUL and PRI cows after the first grazing meal is also commensurate with the greater herbage intake achieved by MUL cows during that meal, stimulating increased BHB production in the liver and in the rumen epithelium (Reynolds, 2002). The increases in glucose and insulin concentrations, which Sheahan *et al*. (2013) found in multiparous dairy cows 2 h after grazing, were not detected in the present study. Possibly, the peak of glucose and insulin had occurred earlier during the grazing meal, explained by the greater intake rate observed. The decrease in glucose concentration in both parities and the slight increase in insulin post-grazing is in agreement with the hypothesis that an increase

in blood BHB may be affecting glucose utilization by peripheral tissues (Krehbiel *et al*., 1992) and/or hepatic glucose production (Schlumbohm and Harmeyer, 2004).

Interestingly, the positive correlations between post-grazing BHB concentration, herbage DM intake and intake rate during the first grazing meal, supports the idea that blood BHB may play a role in the regulation of intake although it should be interpreted with caution and differentiate between centrally and peripherally signaling (Laeger *et al*., 2012). Results of intraperitoneal BHB infusion in goats (Rossi *et al*., 2000) and of the increases of BHB concentration in cerebrospinal fluid in early-lactation dairy cows (Laeger *et al*., 2013) showed that this metabolite was involved in the decreasing of their feed intake in these animals. In addition, the higher post-grazing BHB concentration reached by the MUL cows may suggests different sensitively of this metabolite in cows differing in parity (Laeger *et al*., 2010, Laeger *et al*., 2012). Furthermore, the positive correlation of pre-grazing NEFA concentration with intake rate during the first grazing meal would appear also as a possible orexigenic signal of feed intake.

3.7. CONCLUSION

Where cows' daily access to pasture is limited to 8 h, herbage intake during the first grazing meal comprises 40% of daily herbage intake, and it is during this meal that the largest differences in daily herbage intake between MUL and PRI cows occur. Short-term herbage intake is linked to endocrine-metabolic state and is affected by parity. The NEFA and BHB blood concentrations before and after the first grazing meal appear to be involved in short-term herbage intake regulation. Differences between multiparous and primiparous dairy cows may be explained by different mechanisms, in which the sensitively or threshold level of these metabolites, centrally and/or peripherally, could be involved. Further studies are needed to explain the complex interaction of ruminal, metabolic and hormonal mechanisms involved in grazing intake control in dairy cows.

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3.9. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

3.10. REFERENCES

AOAC (2000) ´Official methods of analysis.´ 17th edn. (Association of Official Analytical Chemists: Gaithersburg, MD)

Astessiano, A.L., Meikle, A., Fajardo, M., Gil, J., Mattiauda, D.A., Chilibroste, P., Carriquiry, M., 2015. Metabolic and endocrine profiles and hepatic gene expression of Holstein cows fed total mixed ration or pasture with different grazing strategies during early lactation. Acta Vet. Scand. 57, 1-12. http://doi.org/10.1186/s13028-015-0163-6

Beauchemin, K.A., Rode, L.M., 1994. Compressed Baled Alfalfa Hay for Primiparous. J. Dairy Sci. 77 (4), 1003-1012. http://doi.org/10.3168/jds.S0022-0302(94)77036-3

Bowman, G.R, Beauchemin, K.A., Shelford, J.A., 2003. Fibrolytic enzymes and parity effects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. J. Dairy Sci. 86, 565-575. http://doi.org/10.3168/jds.s0022-0302(03)73635-2

Chelikani, P.K., Ambrose, J.D., Keisler, D.H., Kennelly, J.J., 2004. Effects of short-term fasting on plasma concentrations of leptin and other hormones and metabolites in dairy cattle. Domest. Anim. Endocrinol. 26, 33-48. http://doi:1.1016/j.domaniend.2003.08.003

Chilibroste, P., Soca, P., Mattiauda, D.A., Bentancur, O., Robinson, P.H., 2007. Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. Aust. J. Exp. Agric. 47, 1075-1084. http://doi.org/10.1071/EA06130

Chilibroste, P., Mattiauda, D.A., Bentancur, O., Soca, P., Meikle, A., 2012. Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. Anim. Feed Sci. Technol. 173, 201-209. http://doi.org/10.1016/j.anifeedsci.2012.02.001

Chilibroste, P., Gibb, M., Soca, P., Mattiauda, D.A., 2015. Behavioural adaptation of grazing dairy cows to changes in feeding management: Do they follow a predictable pattern? Anim. Prod. Sci. 55, 328-338. http://dx.doi.org/10.1071/AN14484

Cissé M, Chilliard Y, Coxam V, Davicco MJ, Rémond B (1991) Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 2. Plasma hormones and metabolites. J. Dairy Sci. 74, 1382-1394. https://doi.org/10.3168/jds.S0022-0302(91)78293-3

Dado, R.G., Allen, M.S., 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. J. Dairy Sci. 77**,** 132-144. https://doi.org/10.3168/jds.S0022-0302(94)76936-8

DIEA (Dirección de Estadísticas Agropecuarias), 2018. Anuario estadístico agropecuario 2018. https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2018/Anuario_2018.pdf. (accessed 16 September 2019).

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68-78. http://doi.org/10.3168/jds.s0022-0302(89)79081-0

Gibb, M., 2007. Grassland management with emphasis on grazing behavior, in: Elgersma, A., Dijkstra, J., Tamminga, S. (Eds.), Fresh herbage for dairy cattle. Springer, Wageningen, pp. 141-157.

Gregorini, P., Soder, K.J., Kensinger, R.S., 2009. Effect of ruminal fill on short-term ingestive behavior and circulating concentrations of ghrelin, insulin and glucose of dairy cows foraging vegetative micro-swards. J. Dairy Sci. 92, 2095-2105. https://doi.org/10.3168/jds.2008-1803

Gregorini, P., Villalba, J., Chilibroste, P., Provenza, F.D., 2017. Grazing management: setting the table, designing the menu and influencing the diner. Anim. Prod. Sci. 57**,** 1248-1269. http://dx.doi.org/10.1071/AN16637

Illius, A.W., Gordon, I.J., 1987. The Allometry of Food Intake in Grazing Ruminants. J. of Anim. Ecol. 56 (3), 989-999. https://doi.org/10.2307/4961

Jones, D.I., Moseley, G., 1993. Laboratory methods for estimating nutritive quality, in: Davies, A., Baker, R.D., Grant, S.A., Laidlaw, A.S. (Eds.), Sward Measurement Handbook (2nd edn). British Grassland Society, Reading, pp. 265-283.

Krehbiel, C.R., Harmon, D.L, Schnieder, J.E., 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux. J. of Anim. Sci. 70, 904-914. http://doi.org/10.2527/1992.703904x

Laeger, T., Metges, C.C., Kuhla, B., 2010 Research review: Role of b-hydroxybutyric acid in the central regulation of energy balance. Appetite 54(3), 450-455. http://doi.org/10.1016/j.appet.2010.04.005

Laeger, T., Pöhland, R., Metges, C.C., Kuhla, B., 2012. The ketone body β-hydroxybutyric acid influences agouti-related peptide expression via AMP-activated protein kinase in hypothalamic GT1-7 cells. J. of Endocrinol. **213**(2), 193-203. http://doi.org/10.1530/JOE-11-0457

Laeger, T., Sauerwein, H., Tuchscherer, A., Bellmann, O., Metges, C.C., Kuhla, B., 2013. Concentrations of hormones and metabolites in cerebrospinal fluid and plasma of dairy cows during the periparturient period. J. Dairy Sci., 96(5), 2883–2893. http://doi.org/10.3168/jds.2012-5909

Lafontan, M., Sengenes, C., Moro, C., Galitzky, J., Berlan, M., 2009. Natriuretic peptides and other lipolytic peptides involved in the control of lipid mobilization, in: Frühbeck, G. (Ed), Peptides in Energy Balance and Obesity. CAB Int., Oxfordshire, pp. 133-161.

Licitra, G., Hernandez, T.M., Van Soest, P., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 47-358. https://doi.org/10.1016/0377-8401(95)00837-3

Maekawa, M., Beauchemin, K.A., Christensen, D.A., 2002. Chewing Activity, Saliva Production, and Ruminal pH of Primiparous and Multiparous Lactating Dairy Cows. J. Dairy Sci. 85, 1176-1182. https://doi.org/10.3168/jds.s0022-0302(02)74180-5

Mattiauda, D.A., Tamminga, S., Gibb, M.J., Soca, P., Bentancur, O., Chilibroste, P, 2013. Restricting access time at pasture and time of grazing allocation for Holstein dairy cows: Ingestive behaviour, dry matter intake and milk production. Livest. Sci. 152, 53-62. http://doi.org/10.1016/j.livsci.2012.12.010

Nikkhah. A., 2012. Timing of feed presentation entrains periprandial rhythms of energy metabolism indicators in once-daily fed lactating cows. Biol. Rhythm Res. 43, 651-666. http://doi.org/10.1080/09291016.2011.631773

NRC (National Research council), 2001. Nutrient requirements of dairy cattle, seventh revised edition. National Academies Press: Washington, DC.

Parker, W.J., McCutcheon, S.N., Carr, D.H., 1989. Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. New Zeal J. Agr. Res. 32, 537-546. http://doi.org/10.1080/00288233.1989.10417928

Penning, P.D., Hooper, G.E., 1985. An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. Grass Forage Sci. 40, 79-84. http://doi.org/10.1111/j.1365-2494.1985.tb01722.x

Pereira, I., Cruz, I., Ruprechter, G., Meikle A., 2017. Salud y eficiencia reproductiva de vacas lecheras en sistemas de base pastoril de Florida: resultados preliminares del monitoreo, in: Matto, C., Delpiazzo, R. (Eds.), XLV Jornadas Uruguayas de Buiatría. Gráfica Mosca, Montevideo, pp. 127-139.

Pérez-Ramírez, E., Delagarde, R., Delaby, L., 2008. Herbage intake and behavioural adaptation of grazing dairy cows by restricting time at pasture under two feeding regimes. Animal. 2(9), 1384-1392. http://doi.org/10.1017/S1751731108002486

Peyraud, J.L., Comeron, E.A., Wade, M.H., Lemaire, G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. Annales de zootechnie 45, 201-217. https://doi.org/10.1051/animres:19960301

Peyraud, J.L., 1998. Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. Techniques for investigating intake and ingestive behaviour by farm animals, in: Davies, A.M.C., Giangiacomo, R. (Eds.), Proceedings of the 9th European intake workshop. IGER, North Wyke, pp. 39-48.

Phillips, C.J., Rind, M.I., 2001. The Effects on Production and Behavior of Mixing Uniparous and Multiparous Cows. J. Dairy Sci. 84, 2424-2429. http://doi.org/10.3168/jds.s0022-0302(01)74692-9

Reynolds, C.K., 2002. Economics of visceral energy metabolism in ruminants: toll keeping or internal revenue service? J. of Anim. Sci. 80(2), 74-84. https://doi.org/10.2527/animalsci2002.80E-Suppl_2E74x

Rossi, R., Dorig, S., Del Prete, E., Scharrer, E., 2000. Suppression of feed intake after parenteral administration of D-beta-hydroxybutyrate in pygmy goats. J. Vet. Med. A. Physiology Pathology Clinical Medicine 47, 9-16. http://doi.org/10.1046/j.1439-0442.2000.00255.x

Sales, J., Janssens, G., 2003. Acid-insoluble ash as a marker in digestibility studies: a review. J. Anim. Feed Sci. 12, 383-401. http://doi.org/10.22358/jafs/67718/2003

Schlumbohm, C and J, Harmeyer., 2004. Hyperketonemia impairs glucose metabolism in pregnant and nonpregnant ewes. J. Dairy Sci. 87, 350-358. http://dx.doi.org/10.3168/jds.S0022-0302(04)73174-4

Sheahan, A.J., Boston, R.C., Roche, J.R., 2013. Diurnal patterns of grazing behavior and humoral factors in supplemented dairy cows. J. Dairy Sci. 96, 3201-3210. http://doi.org/10.3168/jds.2012-6201

Taweel, H.Z., Tas, B.M., Dijkstra, J., Tamminga, S., 2004. Intake Regulation and Grazing Behavior of Dairy Cows Under Continuous Stocking. J. Dairy Sci. 87, 3417-3427. http://doi:10.3168/jds0022-0302(04)73477-3

Tyrrell, H.F., Reid, J.T., 1965. Prediction of the energy value of cows' milk. J. Dairy Sci. 48, 1215-1233. http://doi.org/10.3168/jds.S0022-0302(65)88430-2

Van Soest, P.J., Robertson, J.B., Lewis, B.A.,1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597. http://doi.org/10.3168/jds.S0022-0302(91)78551-2

Weber, C., Hametner, C., Tuchscherer, A., Losand, B., Kanitz, E., Otten, W., Singh, S.P., Bruckmaier, R.M., Becker, F., Kanitz, W., Hammon, H.M., 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96, 165-180. http://doi.org/10.3168/jds.2012-5574

4. DISCUSIÓN Y CONCLUSIONES GENERALES

4.1. DISCUSIÓN

Los experimentos realizados para el presente trabajo fueron diseñados para evaluar el efecto de la dieta (DTM *vs.* pastoreo + suplementación; E1) y el efecto de la paridad en pastoreo (multíparas=MUL vs. primíparas=PRI; E2) en el CMS y comportamiento ingestivo diario, así como en el CMS y comportamiento ingestivo en el primer evento de consumo diario y algunos factores metabólico-hormonales involucrados en su regulación. Como se observó en ambos experimentos, tanto la dieta como la paridad afectaron el comportamiento ingestivo y por lo tanto el CMS, al igual que las señales involucradas en la regulación del primer evento de pastoreo del día.

El CMS y la producción de leche en ambos experimentos fue acorde a la etapa de lactancia y estrategias de alimentación utilizadas. No obstante, tanto la dieta como la paridad afectaron significativamente el CMS. Las vacas alimentadas con DTM presentaron mayores CMS (19,8%) que las vacas en pastoreo (E1), consistente con lo reportado por Bargo *et al.* (2002) quienes compararon vacas lecheras en similares estrategias de alimentación. Por otro lado, las vacas MUL presentaron mayores CMS de forraje (17,5%) que las vacas PRI (E2), al igual que lo reportado por otros autores quienes evaluaron vacas multíparas y primíparas en pastoreo (Peyraud *et al.*, 1996) o estabuladas con DTM (Nikkhah *et al.*, 2011).

El tiempo de observación visual del comportamiento ingestivo fue similar en ambos experimentos (9,4 *vs.* 8 h; E1 y E2, respectivamente); sin embargo, las vacas del E1 presentaron menor tiempo de consumo total que las vacas del E2, distribuido en mayor número de eventos de consumo de menor duración. Estas diferencias podrían estar explicadas por el distinto tiempo de acceso al alimento (16 vs. 8 h; E1 *vs.* E2, respectivamente), lo cual afectó directamente el tiempo de ayuno. Chilibroste *et al*. (2007) reportan cambios en la estrategia de pastoreo asociada a períodos de ayuno, el cual modifica el estado fisiológico de los animales y la sensación de hambre. Posiblemente, las vacas del E1 con mayor tiempo de acceso al alimento, realizaron alguna actividad intensa de consumo inmediatamente luego de la puesta del sol

(post-período de observación), manteniendo el comportamiento ingestivo normal descrito en la bibliografía (Rook *et al.*, 1994; Gibb *et al.*, 1997). En este sentido, las vacas con menor tiempo de acceso al alimento (E2) parecerían desfasar el tiempo de descanso y rumia hacia horas de la noche en pos de incrementar el CMS mediante eventos de consumo más largos e intensos.

Independientemente de la dieta o paridad, las vacas en cada experimento presentaron similar relación entre el CMS en el primer evento y el CMS total, al igual que lo observado con el consumo de NE_L. Esto podría sugerir que los factores involucrados en la regulación del CMS en el primer evento diario podrían ser los mismos que intervienen en la regulación del CMS total. La tasa de consumo en el primer evento parecería ser un factor determinante del CMS y NE_L en dicho evento, principalmente por el similar tiempo de consumo entre tratamientos de cada experimento. En el E1, las vacas alimentadas con DTM presentaron mayor tasa de consumo que las vacas en pastoreo, posiblemente asociada a un mayor peso de bocado (aunque no fue medido) debido al mayor contenido de MS de la DTM respecto a la pastura. En el E2, las vacas MUL presentaron mayor tasa de consumo de forraje que las vacas PRI, asociado también a un mayor peso de bocado. Las vacas MUL presentaron mayor tamaño de arcada que las vacas PRI, lo cual está altamente relacionado al área de bocado, principal variable en afectar el peso del bocado (Gregorini *et al*., 2009). No obstante, como fue discutido en el capítulo (3, E2), las vacas MUL parecerían ajustar el peso de bocado a lo largo del día, asociado al llenado ruminal (Gregorini *et al*., 2009). De este modo, los factores que determinaron el mayor peso de bocado en MUL que en PRI no están claramente identificados, por lo cual factores metabólicos y hormonales también podrían jugar un rol en la regulación de la tasa de consumo a través de cambios en el estado interno y en la sensación de hambre (Chilibroste *et al*., 2015).

Tanto en el E1 como en el E2, las concentraciones de hormonas y metabolitos en sangre (pre- y post-consumo del primer evento diario) fueron acordes con lo reportado por otros autores (Sheahan *et al*., 2013; Wylie *et al*., 2008; Nikkhah, 2014). Previo al primer evento de consumo, las vacas del E1 sólo difirieron en la

concentración de NEFA, mientras que las del E2 sólo difirieron en la concentración de BHB. La similar concentración de las restantes hormonas y metabolitos previo al primer evento de consumo es consistente con el ayuno natural durante la noche y la intensificación de la actividad de rumia (Forbes, 1995; Taweel *et al*., 2004), favoreciendo la reducción del pool ruminal e incrementando la tasa de pasaje y la absorción de nutrientes (Tóthi *et al*., 2003). Luego del consumo, la disminución observada en la concentración de NEFA en las vacas de ambos experimentos, muestra la captación de los nutrientes consumidos durante el evento y el cambio en el estado metabólico de los tejidos, de catabolismo a anabolismo (Lafontan *et al*., 2009). En el mismo sentido, el incremento post-consumo de la concentración de BHB en las vacas de ambos experimentos reflejó la captación de ácidos grasos volátiles durante el evento de consumo (Chilibroste *et al*., 1998). Sin embargo, la tasa de producción de AGV, y el tipo de AGV producido durante el evento podría marcar las diferencias entre las vacas alimentadas con DTM y las vacas en pastoreo.

Las vacas alimentadas con DTM mostraron una caída significativa en la concentración de glucosa en sangre, explicada por un aumento post-consumo de la relación insulina:glucagón, lo cual incrementa la captación de glucosa por los tejidos periféricos (Derno *et al*., 2013). La secreción de estas hormonas está afectada, entre otros, por la variación de la concentración sanguínea de AGV, principalmente propionato (Bines *et al.*, 1983) y la tasa de síntesis de glucosa (Roche *et al*., 2008). El propionato es el principal precursor neoglucogénico de la dieta, y es captado rápidamente de la sangre por el hígado. Esto sugiere que, durante el evento de consumo, un alto flujo de propionato al hígado pudo haber ocurrido en las vacas alimentadas con DTM. Además, el propionato es el principal precursor anapleurótico de la dieta, aportando oxalacetato para la reacción de la citrato sintasa, estimulando la oxidación de acetyl-CoA en el ciclo de Krebs y la producción de ATP a partir del pool de equivalentes de reducción (Allen *et al.*, 2009). En este sentido, el incremento hepático de la relación ATP:ADP post-consumo de las vacas alimentadas con DTM y la correlación negativa observada entre la relación ATP:ADP y la concentración de acetil-CoA post-consumo podrían asociarse a la oxidación hepática de nutrientes. La

tasa de oxidación del propionato en el hígado pudo haberse favorecido por el incremento post-consumo de la relación insulina:glucagón (Derno *et al.*, 2013), soportado además por la correlación positiva entre la relación ATP:ADP y la relación insulina:glucagón encontrada. El incremento en la relación ATP:ADP refleja una mayor tasa de producción de ATP que de utilización. Consistente con la teoría de la oxidación hepática, la señal de saciedad generada en el hígado durante un evento de consumo parecería estar más relacionada al incremento en la tasa de producción de ATP sobre la tasa de utilización y no al incremento de la concentración de ATP *per se* (Allen y Piantoni, 2013; Allen, 2014). A diferencia, en las vacas en pastoreo no se detectaron cambios en la relación ATP:ADP post-consumo como señal de saciedad.

Independientemente del efecto dieta o paridad, es interesante observar que las concentraciones post-consumo de BHB se asociaron a mayores CMS en el primer evento, resaltando la importancia de este metabolito en la regulación del consumo. Además, las correlaciones positivas entre CMS en el primer evento y la concentración post-pastoreo de BHB observadas en el E2 (MUL y PRI juntas) refuerzan esta observación. Algunos autores sugieren que el BHB puede actuar como agente anorexigénico actuando a nivel del hipotálamo (Laeger *et al*., 2010). Otros autores asocian el efecto inhibitorio del BHB en el CMS a la energía generada por la oxidación del BHB en los enterocitos. Por lo tanto, distinta sensibilidad de los tejidos o permeabilidad de la membrana hematoencefálica a este metabolito, podría estar afectada por el tipo de dieta, etapa de lactancia y/o paridad, explicando las diferencias observadas en ambos experimentos.

4.2. CONCLUSIONES

La oxidación hepática de nutrientes no parecería jugar un rol dominante en la regulación del CMS en el corto plazo en vacas en pastoreo. La paridad afectó el estado metabólico animal y este se vio asociado al comportamiento ingestivo y CMS de forraje. La concentración pre-consumo de NEFA y post-consumo de BHB estuvieron involucradas en la regulación del CMS en el corto plazo de vacas multíparas y primíparas en pastoreo y las diferencias entre paridad pueden explicarse por diferentes mecanismos, en los cuales podría estar involucrado el nivel de

sensibilidad de los tejidos a estos metabolitos. Se requieren más estudios para entender la compleja interacción entre los mecanismos endócrino-metabólicos involucrados en la regulación del CMS en el corto plazo de vacas en pastoreo.

5. BIBLIOGRAFÍA

Allden W, Whittaker A. 1970. The determinants of herbage intake by grazing sheep: the interrelationship of factors influencing herbage intake and availabaility. Australian Journal of Agricultural Research, 21: 755 – 766.

Allen MS. 2014. Drives and limits to feed intake in ruminants. Animal Production Science, 54: 1513 – 1524.

Allen MS, Bradford BJ. 2012. Control of food intake by metabolism of fuels: a comparison across species. The Proceedings of the Nutrition Society, 71(3): 401 – 409.

Allen MS, Bradford BJ. 2009. Nutritional control of feed intake in dairy cattle. Ruminant Nutrition Symposium, 20: 138 – 148.

Allen MS, Bradford BJ, Oba M. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of Animal Science, 87(10): 3317 – 3334.

Allen MS, Bradford BJ, Harvatine KJ. 2005. The cow as a model to study food intake regulation. Annual Review of Nutrition, 25(1): 523 – 547.

Allen MS. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science, 83: 1598 – 1624.

Anil MH, Forbes JM. 1988. The roles of hepatic nerves in the reduction of food intake as a consequence of intraportal sodium propionate administration in sheep. Quarterly Journal of Experimental Physiology, 73: 539 – 546.

Anil MH, Forbes JM. 1980. Feeding in sheep during intraportal infusions of short-chain fatty acids and the effect of liver denervation. Journal of Physiology, 298: 407 – 414.

Baile CA, Forbes JM. 1974. Control of Feed Intake and Regulation of Energy Balance in Ruminants. American Physiological Society, 54(1): 160 – 214.

Ballard FJ, Hanson RW, Kronfeld DS, Raggi F. 1968. Metabolic changes in liver associated with spontaneous ketosis and starvation in cows. Journal of Nutrition, 95: 160 – 173.

Bargo F, Muller LD, Delahoy JE, Cassidy TW. 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. Journal of Dairy Science, 85(7): 1777 – 1792.

Barletta R, Gandra J, Bettero V, Araujo E, Cybelle E, Del TA, Gustavo V, Ferreira E, Rodolfo DJ, Bruna DM. 2016. Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows: oilseed provides ruminal protection for fatty acids. Animal Feed Science and Technology, 219: 111 – 121.

Bines JA, Hart IC, Morant SV. 1983. Endocrine control of energy metabolism in the cow: diurnal variations in the concentrations of hormones and metabolites in the blood plasma of beef and dairy cows. Hormone and Metabolic Research, 15: 330 – 334.

Bradford BJ, Allen MS. 2007. Depression in feed intake by a highly fermentable diet is related to plasma insulin concentration and insulin response to glucose infusion. Journal of Dairy Science, 90: 3838 – 3845.

Cabrera Estrada JI, Delagarde R, Faverdin P, Peyraud JL. 2004. Dry matter intake and eating rate of grass by dairy cows is restricted by internal, but not external water. Animaly Feed Science and Technology, 114: 59 – 74.

Chilibroste P, Gibb MJ, Soca P, Mattiauda DA. 2015. Behavioural adaptation of grazing dairy cows to changes in feeding management: Do they follow a predictable pattern?. Animal Production Science, 55: 328 – 338.

Chilibroste P, Mattiauda DA, Bentancur O, Soca P, Meikle A. 2012. Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. Animal Feed Science and Technology, 173: 201 – 209.

Chilibroste P, Soca P, Mattiauda DA, Bentancur O, Robinson PH. 2007. Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. Australian Journal of Experimental Agriculture, 47(9): 1075 – 1084.

Chilibroste P, Tamminga S, Boer H, Gibb MJ, den Dikken G. 2000. Duration of regrowth of ryegrass (Lolium perenne) effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. Journal of Dairy Science, 83: 984 – 995.

Chilibroste P. 1999. Grazing time: the missing link. A study on the plant-animal interface by integration of experimental and modeling approaches. Ph.D. Diss. Wageningen, The Netherlands. Wageningen University. 190 p.

Chilibroste P, Tamminga S, Van Bruchem J, Van der Togt P. 1998. Effect of allowed grazing time, inert rumen bulk and lenght of starvation before grazing on the weight, composition and fermentative end-products of the rumen contents of lactacting dairy cows. Grass and Forage Science, 53: 146 – 156.

Chilibroste P, Tamminga S, Boer H. 1997. Effects of length of grazing session, rumen fill and starvation time before grazing on dry-matter intake, ingestive behaviour and dry-matter rumen pool sizes of grazing lactating dairy cows. Grass and Forage Science, 52(3): 249 – 257.

De Koster JD, Opsomer G. 2013. Insulin resistance in dairy cows. Veterinary Clinics of North America: Food Animal Practice, 29(2), 299 – 322.

Decruyenaere V, Buldgen A, Stilmant D. 2009. Factors affecting intake by grazing ruminants and related quantification methods: a review. Biotechnology, Agronomy, Society and Environment, 13: 559 – 573.

Derno M, Nürnberg G, Schön P, Schwarm A, Röntgen M, Hammon HM, Metges CC, Bruckmaier RM, Kuhla B. 2013. Short-term feed intake is regulated by macronutrient oxidation in lactating Holstein cows. Journal of Dairy Science, 96(2): 971 – 980.

Di Bella J, Tarozzi G, Rossi MT, Scalera G. 1981. Behavioral patterns proceeding from liver thermoreceptors. Physiology and Behavior, 26: 53 – 59.

Edmonson A, Lean I, Weaver L, Farver T, Webster G. 1989. A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science, 72: 68 – 78.

Elliot JM, Symonds HW, Pike B. 1985. Effect on feed intake of infusing sodium propionate or sodium acetate into a mesenteric vein of cattle. Journal of Dairy Science, 68: 1165 – 1170.

Forbes JM. 2007. A personal view of how ruminant animals control their intake and choice of food: minimal total discomfort. Nutrition Research Reviews, 20: 132 – 146.

Forbes JM. 1996. Integration of regulatory signals controlling forage intake in

ruminants. Journal of Animal Science, 74: 3029 – 3035.

Forbes JM. 1995. Voluntary food intake and diet selection in farm animals. 2nd ed. Wallingford, UK: CAB International.

Gibb MJ, Huckle CA, Nuthall R, Rook AJ. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behavior Science, 52: 269 – 287.

Gibb MJ, Huckle CA, Nuthall R. 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science, 53: 41 – 46.

Gibb MJ, Huckle CA, Nuthall R, Rook AJ. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Science, 52 (3): 309 – 321.

Ginane C, Bonnet M, Baumont R, Revell DK. 2015. Feeding behaviour in ruminants: A consequence of interactions between a reward system and the regulation of metabolic homeostasis. Animal Production Science, 55: 247 – 260.

Gregorini P. 2011. Estado interno. Estímulos que motivan el consumo y ciertas conductas ingestivas de rumiantes en pastoreo. En: Brizuela CA, Cangiano MA. (Eds.). Producción Animal en Pastoreo. Balcarce: INTA. Cap. 11, pp. 513 – 565.

Gregorini P, Soder KJ, Kensinger RS. 2009. Effect of ruminal fill on short-term ingestive behavior and circulating concentrations of ghrelin, insulin and glucose of dairy cows foraging vegetative micro-swards. Journal of Dairy Science, 92: 2095 – 2105.

Gregorini P, Tamminga S, Gunter SA. 2006. Review: Behavior and daily grazing patterns of cattle. Professional Animal Scientist, 22(3): 201 – 209.

Grovum WL. 1995. Mechanism explaining the effects of short chain fatty acids on feed intake in ruminants—osmotic pressure, insulin and glucagon. In: Englehardt WV, Leonhard-Marek S, Breves G, Geisecke D. (Eds.). Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Stuttgart, Germany: Ferdinand Enke Verlag. pp. 173–197.

Hills JL, Wales WJ, Dunshea FR, Garcia SC, Roche JR. 2015. Invited review: An evaluation of the likely effects of individualized feeding of concentrate

supplements to pasture-based dairy cows. Journal of Dairy Science, 98(3): 1363 – 1401.

Ji H, Friedman MI. 1999. Compensatory hyperphagia after fasting tracks recovery of liver energy status. Physiology and Behavior, 68: 181 – 186.

Kennedy E, McEvoy M, Murphy JP, O'Donovan M. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. Journal of Dairy Science, 92(1): 168 – 76.

Kolver ES, Muller LD. 1998. Performance and nutrient intake of high producing holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science, 81(5): 1403 – 1411.

Kuhla B. 2012. Central regulation of feed intake in early lactation. En: International Symposium "Nutritional management in early lactation". (2012, Wageningen, the Netherlands).

Laeger T, Metges CC, Kuhla B. 2010. Role of β-hydroxybutyric acid in the central regulation of energy balance. Appetite, 54(3): 450 – 455.

Lafontan M, Sengenes C, Moro C, Galitzky J, Berlan M. 2009. Natriuretic peptides and other lipolytic peptides involved in the control of lipid mobilization. En: Frühbeck G (Ed.). Peptides in Energy Balance and Obesity. Oxfordshire: CAB Int. pp. 133 – 161.

Langhans W, Damaske U, Scharrer E. 1985. Different metabolites might reduce food intake by the mitochondrial generation of reducing equivalents. Appetite, 6: 143 – 152.

Mayer J. 1953. Glucostatic mechanism of regulation of food intake. The New England Journal of Medicine, 249: 13 – 16.

Mbanya JN, Anil MH, Forbes JM. 1993. The voluntary intake of hay and silage by lactating cows in response to ruminal infusion of acetate or propionate, or both, with or without distension of the rumen by a balloon. British Journal of Nutrition, 69: 713 – 720.

Meikle A, Cavestany D, Carriquiry M, Adrien M. de L, Artegoitia V, Pereira I, Ruprechter G, Pessina P, Rama G, Fernández A, Breijo M, Laborde D, Pritsch O, Ramos JM, De Torres E, Nicolini P, Mendoza A, Dutour J, Fajardo M, Ana,

L, Olazábal L, Mattiauda D, Chilibroste P. 2013. Avances en el conocimiento de la vaca lechera durante el período de transición en Uruguay□: un enfoque multidisciplinario. Agrociencia Uruguay, 17: 141 – 152.

Meikle A. Kulcsar M, Chilliard Y, Febel H, Delavaud C, Cavestany D, Chilibroste P. 2004. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction, 127: 727 – 737.

Niijima A. 1969. Afferent impulse discharges from glucoreceptors in the liver of the guuinea pig. Annals of the New York Academy of Sciences, 157: 690 – 700.

Nikkhah A. 2014. Review: Ruminant feed intake regulation evolution: Chronophysiological rhythms perspectives. Biological Rhythm Research, 45: 563 – 577.

Nikkhah A. 2012. Timing of feed presentation entrains periprandial rhythms of energy metabolism indicators in once-daily fed lactating cows. Biological Rhythm Research, 43: 651 – 66.

Nikkhah A, Furedi CJ, Kennedy AD, Scott SL, Wittenberg KM, Crow GH, Plaizier JC. 2011. Morning vs. evening feed delivery for lactating dairy cows. Journal of Animal Science, 91: 113 – 122.

Oba M, Allen MS. 2003a. Dose-response effects of intrauminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. Journal of Dairy Science, 86: 2922 – 2931.

Oba M, Allen MS. 2003b. Hypophagic effect of ammonium is greater when infused with propionate compared with acetate in lactating dairy cows. Journal of Nutrition, 133: 100 – 1104.

Oba M, Allen MS. 2003c. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. Journal of Nutrition, 133: 1094 – 1099.

Oba M, Allen MS. 2003d. Effects of intraruminal infusion of sodium, potassium, and ammonium on hypophagia from propionate in lactating dairy cows. Journal of Dairy Science, 86: 1398 – 404.

Oba M, Allen MS. 2003e. Extent of hypophagia caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. Journal of

Nutrition, 133: 1105 – 12.

Oba M, Allen MS. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. Journal of Dairy Science, 82: 589 – 596.

Patterson DM, McGilloway DA, Cushnahan A, Mayne CS., Laidlaw AS. 1998. Effect of duration of fasting period on short-term intake rates of lactating dairy cows. Animal Science, 66(2): 299 – 305.

Peyraud JL, Comeron EA, Wade MH, Lemaire G. 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. Annales de Zootechnie, 45: 201 – 217.

Phillips CJ, Rind MI. 2001. The effects on production and behavior of mixing uniparous and multiparous cows. Journal of Dairy Science, 84: 2424 – 2429.

Piantoni P, Ylioja CM, Allen MS. 2015. Feed intake is related to changes in plasma nonesterified fatty acid concentration and hepatic acetyl CoA content following feeding in lactating dairy cows. Journal of Dairy Science, 98: 6839 – 6847.

Rawson NE, Friedman MI. 1994. Phosphate loading prevents the decrease in ATP and increase in food intake produced by 2,5-anhydro-d-mannitol. American Journal of Physiology, 266: 1792–1796.

Remppis S, Steingass H, Gruber L, Schenkel H. 2011. Effects of energy intake on performance, mobilization and retention of body tissue, and metabolic parameters in dairy cows with special regard to effects of pre-partum nutrition on lactation: A review. Asian-Australasian Journal of Animal Sciences, 24: 540 – 572.

Reynolds CK, Aikman PC, Lupoli B, Humphries DJ, Beever DE. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. Journal of Dairy Science, 86: 1201 – 1217.

Roche JR, Blache D, Kay JK, Miller DR, Sheahan AJ, Miller DW. 2008. Neuroendocrine and physiological regulation of intake with particular reference to domesticated ruminant animals. Nutrition Research Reviews, 21(2): 207.

Rook AJ, Huckle CA, Penning PD. 1994. Effect of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. Applied Animal Behaviour Science, 40: 101 – 112.

Russek M. 1963. Participation of hepatic glucoreceptor in the control of intake of food. Nature, 197: 79 – 80.

Santos JEP, DePeters EJ, Jardon PW, Huber JT. 2001. Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows. Journal of Dairy Science, 84: 213 – 224.

Seoane JR, Baile CA, Martin FH. 1972. Humoral factors modifying feeding behavior of sheep. Physiology and Behavior, 8: 993 – 995.

Sheahan AJ, Boston RC, Roche JR. 2013. Diurnal patterns of grazing behavior and humoral factors in supplemented dairy cows. Journal of Dairy Science, 96: 3201 – 10.

Stocks SE, Allen MS. 2012. Hypophagic effects of propionate increase with elevated hepatic acetyl coenzyme A concentration for cows in the early postpartum period. Journal of Dairy Science, 95: 3259 – 3268.

Taweel HZ, Tas BM, Dijkstra J, Tamminga S. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science, 87: 3417 – 3427.

Toates F. 2002. Physiology, motivation and the organization of behaviour. En: Jensen P. (Ed.). The ethology of domestic animals: an introductory text. Wallingford, UK: CAB International. 31 – 50.

Tóthi R, Zhang RH, Chilibroste P, Boer H, Tamminga S. 2003. Effect of processed cereal grains as a supplement on grass intake, rumen pool sizes, ruminal kinetics and the performance of grazing lactating dairy cows. Journal of Animal and Feed Sciences, 12: 417 – 433.

Wales WJ, Dellow DW, Doyle PT, Egan AR. 2000. Effects of feeding additional pasture hay in autumn to dairy cows grazing irrigated perennial ryegrass-white clover pasture and supplemented with barley grain. Australian Journal of Experimental Agriculture, 40: 1 – 9.

Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, Singh SP,

Bruckmaier RM, Becker F, Kanitz W, Hammon HM. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. Journal of Dairy Science, 96: 165 – 180.

Wylie ARG, Woods S, Carson AF, McCoy M. 2008. Periprandial changes in metabolite and metabolic hormone concentrations in high-genetic-merit dairy heifers and their relationship to energy balance in early lactation. Journal of Dairy Science, 91: 577 – 586.